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**IMPROVING DIAGNOSIS AND
UNDERSTANDING THE
PATHOPHYSIOLOGY OF TUBERCULOUS
MENINGITIS**

by

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A thesis submitted in partial fulfillment of the requirements of The Open
University for the degree of Doctor of Philosophy

Oxford University Clinical Research Unit

Centre for Tropical Medicine

Ho Chi Minh City

Viet Nam

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Abstract

Tuberculous meningitis (TBM) is the most severe form of TB. Current diagnostics are insufficiently sensitive. Processes of excessive neuroinflammation are poorly understood. Detecting raised intracranial pressure is challenging. TBM-associated hyponatraemia is poorly understood. This thesis aims to improve diagnosis of TBM and its complications, and further understand TBM's complex pathophysiology.

Firstly, I present a prospective randomised evaluation of the diagnostic performance of GeneXpert MTB/RIF (Xpert) against GeneXpert MTB/RIF Ultra (Ultra) in 205 individuals with TBM. Diagnostic sensitivities of Ultra and Xpert against a clinical TBM reference standard were 47.2% (25/53, 95% confidence interval [CI] 34.4-60.3%) and 39.6% (21/53, 95% CI 27.6-53.1%) respectively ($p=0.56$).

Next, I present data from two randomised trials of adjunctive dexamethasone in clinical TBM (ACT HIV [NCT03092817] and LAST ACT [NCT0310078]). 668 adults with TBM underwent baseline *S. stercoralis* testing. Active *S. stercoralis* infection significantly associated with reduced median cerebrospinal fluid (CSF) interferon (IFN)- γ , interleukin (IL)-2, and tumour necrosis factor (TNF)- α concentrations (3.51 vs. 5.81pg/mL $p=0.01$; 5.05 vs. 5.77pg/mL $p=0.03$; 2.17 vs. 3.58pg/mL $p=0.02$, respectively), and with reduced neurological complications by 3 months (3.8%[1/26] vs. 30.0%[33/110], respectively, $p=0.01$). In 107 adults with TBM, higher baseline optic nerve sheath diameter (ONSD) associated with more severe TBM and abnormal brain imaging (abnormal imaging 0.55cm vs. normal imaging 0.50cm, $p=0.01$). Baseline ONSD was higher in participants who died by 3 months (0.56cm [15/72]) vs. participants who survived (0.52cm [57/72]), $p=0.02$. Finally, 208 adults with TBM underwent longitudinal testing of plasma sodium, urinary sodium, serum osmolality, or urine osmolality. Baseline plasma sodium significantly associated with higher lumbar CSF opening pressure, and elevated CSF neutrophils. Plasma sodium was significantly lower at all time points after baseline in participants who died by 3 months.

Ultra was not superior to Xpert for TBM diagnosis. However, given this study was powered to detect a 25% improvement in diagnostic sensitivity with Ultra it remains possible that Ultra was more sensitive than Xpert at a lower margin of superiority. *S. stercoralis* co-infection may modulate the intracerebral inflammatory response to *M. tuberculosis*. ONSD ultrasound may identify severity and predict death, in TBM. Persistent hyponatraemia associates with poor clinical outcomes.

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Abbreviations list

ACEP: American College of Emergency Physicians
ADA: adenosine deaminase
ADH: anti-diuretic hormone
AFB: acid-fast bacilli
AIDS: acquired immunodeficiency syndrome
ANP: atrial natriuretic peptide
APC: antigen presenting cell
ART: anti-retroviral therapy
ATT: anti-tuberculosis chemotherapy
BCG: bacillus Calmette-Guerin
BNP: brain natriuretic peptide
CBF: cerebral blood flow
CD: cluster of differentiation
CDC: Centers for Disease Control and Prevention
CFU: colony forming units
CI: confidence interval
CNS: central nervous system
COVID: coronavirus disease
CSF: cerebrospinal fluid
CSW: cerebral salt wasting
CT: computed tomography
CTU: Clinical Trials Unit
CVP: central venous pressure
CY: cytochrome
DILI: drug induced liver injury
DNA: deoxyribonucleic acid
DST: drug susceptibility testing
E: ethambutol
EJE: European Journal of Endocrinology
ELISA: enzyme linked immunosorbent assay
ETV: endoscopic third ventriculostomy
EVD: external ventricular drain
FIND: Foundation for Innovative New Diagnostics

FLAIR: fluid-attenuated inversion recovery
GCS: Glasgow coma score
HIV: human immunodeficiency virus
HTD: Hospital for Tropical Diseases
HTLV-1: human T-lymphotropic virus-1
I: isoniazid
ICP: intracranial pressure
ID: identification
IFN: interferon
IL: interleukin
IRIS: immune reconstitution inflammatory syndrome
IVC: inferior vena cava
LAM: lipoarabinomannan
LAMP: loop-mediated isothermal amplification
LED: light emitting diode
LTA4H: leukotriene A4 hydrolase
MDR: multidrug resistance
MGIT: mycobacteria growth indicator tube
MHC: major histocompatibility complex
MIC: minimum inhibitory concentrations
MODS: microscopic observation drug susceptibility
MRC: Medical Research Council
MRI: magnetic resonance imaging
NAAT: nucleic acid amplification tests
NAT: N-acetyltransferase
NICE: National Institute for Health and Care Excellence
NK: natural killer
NNRTIs: non-nucleoside reverse transcriptase inhibitors
NPV: negative predictive value
OD: optical density
ONS: optic nerve sheath
ONSD: optic nerve sheath diameter
OUCRU: Oxford University Clinical Research Unit
PCR: polymerase chain reaction
PI: protease inhibitor

PPV: positive predictive value
R: rifampicin
RCT: randomised controlled trial
ROC: receiver operating characteristic
S: streptomycin
SD: standard deviation
SDG: sustainable development goal
SIADH: syndrome of inappropriate antidiuretic hormone secretion
SIRE: streptomycin, isoniazid, rifampicin, ethambutol
SNP: single nucleotide polymorphism
TB: tuberculosis
TBI: traumatic brain injury
TBM: tuberculous meningitis
TEN: toxic epidermal necrolysis
TLR: toll-like receptor
TNF: tumour necrosis factor
Ultra: GeneXpert MTB/RIF Ultra
UN: United Nations
VP: ventriculoperitoneal
WBC: white blood cell
WHO: World Health Organization
Xpert: GeneXpert MTB/RIF
ZN: Ziehl-Neelsen

Publications related to this thesis

1. Xpert MTB/RIF Ultra for the diagnosis of tuberculous meningitis: a step forward, but still not good enough

Joseph Donovan, Fiona V. Cresswell, Nguyen Thuy Thuong Thuong, David R. Boulware, Guy E. Thwaites, and Nathan C. Bahr, for the Tuberculous Meningitis International Research Consortium

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2. Tuberculous meningitis – where to from here?

Joseph Donovan, Guy E. Thwaites, Julie Huynh

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3. A randomised comparison of GeneXpert Ultra MTB/RIF and GeneXpert MTB/RIF for the diagnosis of tuberculous meningitis

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The Lancet Infectious Diseases. Jan 2020; 20: 308–17

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5. The neurocritical care of tuberculous meningitis

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Chapter 1

Introduction

1.1 Objectives of this thesis

My thesis is titled ‘Improving diagnosis and understanding the pathophysiology of tuberculous meningitis’. Tuberculous meningitis (TBM) is the most severe form of TB, killing or severely disabling half of those it affects. Whilst promising progress has been made in this research field in recent years, the lack of high quality data to guide best practice is alarming. My objective is to improve diagnosis and better understand pathophysiology in TBM, focusing on important clinical questions which I will outline in the introduction, and on those participants with severe TBM in whom complications occur most frequently. I believe my thesis offers new insights into TBM, and describes new data that can both be used in clinical practice, and taken further forward with new research.

My thesis will be separated into six chapters. Following an introduction to TBM focusing on the general themes to be explored in this thesis, I will include four data chapters; Ultra vs. Xpert (diagnosis), *Strongyloides* immunomodulation (pathogenesis and inflammation), ONSD ultrasound (diagnosis of severity), and hyponatraemia (pathogenesis), followed by a discussion and my conclusions. The individual chapter structure of my thesis is as follows:

1.1.1 Chapter 1

General introduction to TBM

1.1.2 Chapter 2

A prospective randomised evaluation of the diagnostic performance of standard 1st generation GeneXpert MTB/RIF against GeneXpert MTB/RIF Ultra in tuberculous meningitis

Objectives

1. Compare GeneXpert MTB/RIF Ultra with GeneXpert MTB/RIF for the diagnosis of TBM
2. Evaluate the performance of GeneXpert MTB/RIF Ultra and GeneXpert MTB/RIF on anti-tuberculosis chemotherapy

1.1.3 Chapter 3

The influence of *Strongyloides stercoralis* co-infection on the presentation, pathogenesis, and outcome of tuberculous meningitis

Objectives

1. Identify the frequency of *S. stercoralis* co-infection in human immunodeficiency virus (HIV) co-infected and HIV uninfected adults with TBM
2. Compare the performances of available diagnostic tests for *S. stercoralis*; stool microscopy, stool polymerase chain reaction (PCR), and serology
3. Explore the relationship between *S. stercoralis* co-infection, TBM presentation and routine CSF parameters
4. Explore the relationship between *S. stercoralis* co-infection and pre-treatment CSF cytokine concentrations in TBM
5. Explore the relationship between *S. stercoralis* co-infection and clinical outcomes in TBM

1.1.4 Chapter 4

The role of optic nerve sheath ultrasound as a non-invasive tool for intracranial pressure (ICP) monitoring in adults with tuberculous meningitis

Objectives

1. Characterise optic nerve sheath diameter at baseline, by sex, final diagnosis, Medical Research Council (MRC) TBM grade, and HIV co-infection status, in adults with TBM
2. Correlate ONSD with brain imaging consistent with raised ICP, or with abnormal brain imaging appearances
3. Use receiver operating characteristic (ROC) curve analysis to select an ONSD cut-off value that predicts abnormal brain imaging, or death by 3 months, with clinically acceptable sensitivity and specificity
4. Describe the association of non-quantitative optic nerve sheath appearances with brain imaging
5. Describe the association between ONSD and plasma sodium
6. Correlate ONSD with neurological complications by 3 months, and with death by 3 months
7. Describe ONSD trends during the first 30 days of TBM treatment, stratified by HIV co-infection, TBM severity grade, and clinical endpoints

1.1.5 Chapter 5

A descriptive analysis of the pathophysiology of TBM-associated hyponatraemia by serial assessments of plasma and urinary sodium, serum and urinary osmolalities, fluid balance and intravascular volume

Objectives

1. Describe the phenotypic characteristics and laboratory parameters associated with hyponatraemia at TBM presentation
2. Describe the association between baseline plasma sodium, serum osmolality, urinary sodium, urinary osmolality, and plasma cortisol, in TBM
3. Describe the likely processes of hyponatraemia in TBM
4. Describe the progression of sodium and volume parameters during the first 30 days of TBM treatment
5. Describe the influence of baseline plasma sodium, serum osmolality, urinary sodium, urinary osmolality, and plasma cortisol, on clinical outcomes in TBM
6. Describe the corrective sodium therapy used in hyponatraemia of TBM

1.1.6 Chapter 6

Discussion and conclusions

1.2 Research contribution

Below, in line with transparent research practices, I have detailed my contribution to each thesis chapter:

Chapter 1: I planned and performed the literature review for this chapter, incorporating structuring advice from my PhD supervisors, and then wrote the introduction.

Chapter 2: I assisted with case report form design and the setting up of study procedures. I reviewed and helped recruit participants on daily clinical ward rounds. I planned the analysis, performed all interim analyses, and wrote the analysis code using the computing language R. I performed data cleaning, reviewed for and collected missing data, and performed the final analysis. I wrote the manuscript and this chapter.

Chapter 3: I reviewed and helped recruit participants on daily clinical ward rounds. I performed day-to-day management of the two large clinical trials through which these data were collected, ensuring compliance with study protocols and standard operating procedures. I regularly reviewed

study data and monitored the ongoing progress of the research. I arranged and coordinated *S. stercoralis* stool PCR testing in conjunction with Oxford University Clinical Research Unit (OUCRU) emerging infections group who performed this PCR testing. I performed CSF cytokine analysis in conjunction with a colleague from the TB research group with experience in these techniques. I reviewed for and collected, missing data, and cleaned data. I wrote the analysis code using the computing language R and performed the data analysis. I wrote the manuscript and this chapter.

Chapter 4: I reviewed and helped recruit participants on daily clinical ward rounds. I performed day-to-day management of the two large clinical trials through which these data were collected, ensuring compliance with study protocols and standard operating procedures. I regularly reviewed study data and monitored the ongoing progress of the research. I developed skills in point-of-care optic nerve sheath ultrasound and underwent additional training overseas in this technique. I designed a pilot study to ensure accurate performance of ONSD measurement by 2 independent operators and performed >95% of ONSD ultrasounds. I designed data collection, re-designed the case report form, and sought successful ethics approval for the performing of this technique in healthy volunteers. I reviewed for and collected, missing data, and cleaned data. I wrote the analysis code using the computing language R and performed the data analysis. I wrote the manuscript and this chapter.

Chapter 5: I reviewed and helped recruit participants on daily clinical ward rounds. I performed day-to-day management of the two large clinical trials through which these data were collected, ensuring compliance with study protocols and standard operating procedures. I regularly reviewed study data and monitored the ongoing progress of the research. I met with local laboratory collaborators in Ho Chi Minh City to set up testing for serum and urine osmolality (Cho Ray Hospital) and plasma cortisol (Medic Medical Center). I designed data collection, and re-designed the case report form. I regularly reviewed study data, and adherence to clinical trial protocols at the recruiting site. I reviewed for and collected, missing data, and cleaned data. I wrote the analysis code using the computing language R and performed the data analysis. I wrote the manuscript and this chapter.

Chapter 6: I planned and wrote the discussion and conclusions

1.3 An introduction to tuberculous meningitis

1.3.1 Epidemiology

1.3.1.1 Global

According to the most recent Global Tuberculosis (TB) report published by the World Health Organization (WHO), in 2019 an estimated 10 million individuals developed TB-illness, with an estimated 1.5 million TB deaths.[1] TB remains the leading cause of death from a single bacterial infection,[1] yet reducing incident cases and deaths has proven challenging. Given insufficient recent progress towards meeting the ambitious United Nations (UN) sustainable development goal (SDG) of ending the TB epidemic by 2030,[2] in September 2018 a UN high level meeting on TB was held, where political commitments to UN SDGs and to the WHO's End TB strategy were confirmed, and new goals added.[1]

Tuberculous meningitis is the most severe form of TB, leading to death in 30-50% of sufferers despite treatment.[3–6] The global incidence of TBM is uncertain. Limited data suggest TBM represents approximately 1% of all TB cases, leading to an estimated 100,000 global cases per year. In Germany, a study of 46,349 TB cases found TBM in 0.9%,[7] whereas a Brazilian study of 427,548 TB cases found 57,217 extrapulmonary TB cases, with TBM accounting for 6% of these extrapulmonary cases.[8] TBM incidence is expected to vary by geographical location, all-form TB incidence, HIV co-infection rate, and population age structure.[9] Current estimates suggest TBM may in fact account for up to 2-5% of TB cases.[10] TBM can affect any group, but is especially common in young children and in those co-infected with HIV.[9] Prior bacillus Calmette-Guerin (BCG) vaccination appears to offer partial protection against childhood TBM and mortality, potentially through improved cellular immunity and prevention of *Mycobacterium tuberculosis* dissemination.[11–13]

1.3.1.2 Vietnam

The current TB incidence in Vietnam is 182 cases per 100,000 population, resulting in a calculated estimate of 174,000 TB cases in a country of 96 million individuals.[1] Applying 1% and 5% estimates of TBM incidence, an estimated 1740-8700 cases of TBM will occur in Vietnam each year. The proportion of these TBM cases presenting to health facilities in Vietnam is unknown, however those that do, and are correctly identified as TBM, are referred to tertiary referral sites. Additionally, many cases of undiagnosed brain disease are referred to tertiary referral sites where a diagnosis of TBM is then made. In Ho Chi Minh City, Vietnam, tertiary referral sites for TBM are the Hospital for Tropical Diseases (HTD) and Pham Ngoc Thach Hospital for Tuberculosis and Lung Disease, where approximately 100 adult cases of TBM, and 300 adult and 50 paediatric cases of TBM, respectively, are identified and treated each year.

1.3.2 Pathophysiology of TBM

1.3.2.1 Infection and dissemination

M. tuberculosis infection occurs when *M. tuberculosis* is inhaled by a human host, with subsequent infection of alveolar macrophages, formation of a primary complex, and dissemination to regional lymph nodes.[14] In individuals who develop TBM, bacteraemia follows; *M. tuberculosis* bacilli seed to the meninges or brain parenchyma forming small subpial or subependymal granulomas called 'Rich foci'. [14] Rupture of these granulomas into the subarachnoid space remains the accepted theory of TBM pathogenesis, where two sequential foci (pulmonary then central) are required.[9,15] Upon rupture of the central granuloma, *M. tuberculosis* bacilli enter the central nervous system (CNS) and are taken up by microglial cells (in which they can also replicate), leading to release of microglial cytokines and chemokines.[9,16,17]

The resulting inflammatory response to *M. tuberculosis* entering the CNS is variable, and likely dictated by both bacillary and host factors; but tissue damage and disease complications often result. Too little or too much inflammation are both considered detrimental to outcome.[18] The processes of *M. tuberculosis* induced host inflammation within the lung are well researched,[19] however, *M. tuberculosis* induced inflammation within the brain is not fully understood, but may involve activation of the microglial NLRP3 inflammasome, an immune complex of receptors and sensors that mediates innate immune responses and induces inflammation.[17,20]

1.3.2.2 Intracerebral immune responses

Both innate and acquired immune responses are part of the host response to TBM. A pro-inflammatory immune response, largely regulated by CD4+ T cells, and predominated by tumour necrosis factor (TNF)- α , interferon (IFN)- γ , and interleukin (IL)-2 cytokine release, is typical of TBM. When this pro-inflammatory response is dysregulated and excessive, as often occurs, significant brain tissue damage can result.

Neutrophils, long considered effector cells of the innate immune system,[21] appear to be an important factor in TBM-associated inflammation. In a study of HIV uninfected individuals over 14 years old with TBM in Indonesia, higher CSF neutrophil counts were significantly associated with death.[22] In a study of TBM-associated immune reconstitution inflammatory syndrome (IRIS) after commencing anti-retroviral therapy (ART) in South Africa, more severe inflammation was associated with high baseline CSF neutrophil counts.[23] Neutrophils produce both pro- and anti-inflammatory cytokines and chemokines,[24] including TNF- α and IFN- γ ; therefore their role in TBM is likely complex. A variety of CSF pro-inflammatory cytokines are in fact elevated in TBM,

however these cytokine concentrations have shown little correlation with TBM severity or clinical outcome in most previous studies.[25–27] Host directed therapies such as corticosteroids and newer biological agents (e.g. infliximab), used to attempt to control host inflammation, have been used with limited and variable success.[5,28–30] The benefit of therapeutically reducing TNF- α concentrations in TBM remains uncertain.

The presence of CSF lymphocytes, and the elevation of CSF IFN- γ and IL-2 (predominantly produced by T cells), provides evidence of an acquired immune response in TBM. In a prospective CSF flow cytometry study of 67 HIV uninfected individuals more than 14 years of age with definite or probable TBM in Indonesia, CSF analysis showed a predominance of $\alpha\beta$ T cells (a T cell group inclusive of subsets displaying either the CD4+ or CD8+ receptor), alongside variable proportions of natural killer cells and neutrophils.[31] Cellular release of IFN- γ , upon clinical sample stimulation with mycobacterial antigens, forms the basis of IFN- γ release assay testing. This shows a clear acquired immune response to mycobacteria, and this testing has been described for CSF in TBM.[32]

1.3.2.3 Factors influencing intracerebral inflammatory response

1.3.2.3.1 Human immunodeficiency virus

HIV co-infection increases mortality from TBM, compared with HIV uninfected individuals.[33] Amongst HIV co-infected individuals with TBM, lower peripheral CD4+ cell counts and lower CSF lymphocyte counts predict mortality at 9 months,[34,35] suggesting reduced immunological response to TBM is detrimental to outcome. In HIV co-infected TBM, *M. tuberculosis* identification by Xpert is higher than in HIV uninfected individuals[36,37] consistent with higher CSF *M. tuberculosis* bacillary loads in HIV co-infection. This indicates that increased bacterial replication is possible in HIV co-infection; however, patient factors (e.g. later presentation) or bacillary factors (e.g. increased virulence or drug resistance) must be considered as potential confounders. In a study of pre-treatment intracerebral inflammation in TBM from Vietnam, HIV co-infection was significantly associated with higher CSF neutrophil proportion and a globally increased production of CSF cytokines.[18] Taken together, this supports more *M. tuberculosis* bacilli and more intracerebral inflammation in HIV co-infected TBM, with both increased CSF neutrophils and an impaired T cell response contributing to worse outcomes.

1.3.2.3.2 Host genetics

Host (i.e. human) genetic polymorphisms may influence susceptibility and response to infection with *M. tuberculosis*. In 2010 a zebrafish model demonstrated that the *leukotriene A4 hydrolase* (*LTA4H*) gene influenced the balance of pro and anti-inflammatory eicosanoids in response to *M. tuberculosis* infection.[38] *LTA4H* catalyses the final step in pro-inflammatory leukotriene B4 (LTB4) synthesis,[38] with the effects of LTB4 balanced by anti-inflammatory lipoxin A4 (LXA4); the two together regulating an inflammatory response to *M. tuberculosis* without excessive tissue damage.[39] A single nucleotide polymorphism (SNP) (rs17525495) in the promoter region of the *LTA4H* gene alters the gene's expression, and the critical LTB4:LXA4 balance; low (CC) and high (TT) inflammatory states result from *LTA4H* allele homozygosity whereas an intermediate (CT) inflammatory state results from allele heterozygosity.[38] Both TT and CC inflammatory states were associated with an increased risk of death in a retrospective adult TBM study.[40] In this retrospective study adjunctive dexamethasone improved survival in the high inflammatory TT group, with the effect of dexamethasone unclear in the CC and CT groups.[40] The influence of *LTA4H* genotype upon adjunctive corticosteroid therapy is now being evaluated in an ongoing randomised placebo-controlled *LTA4H* genotype stratified non-inferiority trial of HIV uninfected adults with TBM in Vietnam (NCT03100786).[41] If the benefit of adjunctive corticosteroids is limited to one (or more) *LTA4H* genotypes, this potentially paves the way for personalised corticosteroid therapy in TBM.

Additionally, an SNP (rs17842268) in CD43, (a cell surface glycoprotein), has been associated with more severe presentation, and decreased survival, in TBM.[42] Why CD43 SNPs impact upon TBM this way is uncertain, however, CD43 does have a role in regulating proinflammatory cytokines,[42] suggesting immunomodulation as the mechanism. The CSF metabolome of TBM, with these downstream metabolomic markers influenced by host genotype, may reveal new genes important for survival in TBM. In a study of 33 HIV uninfected individuals with TBM, plus 22 control individuals, lower CSF tryptophan (one such metabolite) was associated with improved survival.[43] Tryptophan affects *M. tuberculosis* growth and CNS inflammation, and its production and/or release are under genetic influence.

1.3.2.3.3 Bacillary genetics

Broadly, *M. tuberculosis* complex (i.e. the group of closely related mycobacteria causing TB, including *M. tuberculosis*) can be separated into 8 phylogenetic lineages affecting humans.[44,45] The predominant lineage in Vietnam is the Beijing lineage (lineage 2); this lineage appears more transmissible between hosts, and has been increasing its proportion of all TB lineages in Vietnam over the past 10 years.[46] Genetic variation (insertions, deletions, rearrangement, or point

mutations) between *M. tuberculosis* strains allows for tracking of a particular strain, thereby improving the understanding of TB spread and pathogenicity.[47] A variety of bacillary mutations and virulence factors have been described for *M. tuberculosis*.[48,49]

In an in-vitro study of *M. tuberculosis* isolates obtained from children in South Africa, *M. tuberculosis* growth patterns and host cytokine production (TNF and IL12p40) were different between *M. tuberculosis* lineages 2, 3, and 4.[50] It follows that different strains may evoke different CSF cytokine profiles in TBM; however limited data are available describing bacillary genetics and impact upon clinical TBM. In a retrospective lineage study of 222 Vietnamese individuals > 14 years of age with TBM, the Beijing lineage was significantly associated with HIV co-infection, and with multidrug resistance.[51] In an additional study from the same group comparing bacillary and host genetics of 187 adults with TBM vs. 237 non-disseminated pulmonary TB cases, infection with Euro-American (lineage 4) isolates resulted in lower mortality.[52] Additionally, a specific polymorphism in the host toll-like receptor (TLR)-2 gene was associated with TBM disease caused by the Beijing lineage. The Beijing lineage is considered an aggressive TB lineage in relation to its global spread and association with treatment failure. Yet, in a study of 186 HIV co-infected adults with TBM in Vietnam, the Beijing lineage was associated with reduced mortality compared with the Indo-Oceanic lineage (lineage 1). Effects of bacillary genetics on intracerebral inflammation remain poorly understood, and the interaction of both host and bacterial factors is likely more important than bacillary lineage or specific bacillary mutations alone.

1.3.2.3.4 Helminth co-infection

The soil transmitted helminth *Strongyloides stercoralis* is a neglected tropical disease endemic to Vietnam. Helminths such as *S. stercoralis* induce downregulation of pro-inflammatory immunity, with predominance of eosinophils, and IgE antibody production. In TBM with *S. stercoralis* co-infection pro-inflammatory immune responses of TBM may therefore be downregulated by helminth co-infection. In a pulmonary TB study, plasma IFN- γ , TNF- α , and IL-2 were reduced in TB *S. stercoralis* co-infection vs. TB alone.[53] Whether helminth co-infection detrimentally impairs immune response to TBM, or minimises harmful and excessive pro-inflammatory immune responses of TBM, will be described for the first time in this thesis (chapter 3).

1.3.3 Clinical presentation of TBM

TBM classically presents as a subacute meningitic illness,[15] with onset of headache, neck stiffness, confusion and fever over many days or weeks. This indolent presentation may delay presentation to hospital, and delayed treatment may reduce chances of a good clinical outcome.[15]

Early clinical presentation in children can be non-specific, with vomiting, malaise and failure to thrive.[15] More severe presentations of TBM include reduced consciousness and/or focal neurological signs, often as a result of raised ICP, with an urgent need for interventional neurocritical care.

TBM severity is classified by the modified British MRC TBM severity grading, originally developed in 1948 and subsequently modified after the introduction of the Glasgow coma score (GCS).[9] The currently used MRC TBM severity grading is shown in table 1-1. Rates of mortality and severe neurological disability increase as TBM grade increases, and HIV co-infection increases mortality further.[9]

Table 1-1: Modified MRC TBM severity grades

TBM grade	GCS and neurological signs
Grade I	GCS 15; no focal neurological signs
Grade II	GCS 11-14, or 15 with focal neurological signs
Grade III	GCS \leq 10 with or without focal neurological signs

GCS=Glasgow coma score. MRC=Medical Research Council. TBM=tuberculous meningitis.

The clinical presentation of TBM may be mistaken for one of many non-TB meningitides, which share symptoms, signs, and CSF parameter patterns with TBM. A history of symptoms of more than 5 days usually excludes bacterial meningitis, supported by a high (but not excessively high) CSF white blood cell (WBC) count that typically is predominantly lymphocytic. Viral meningoencephalitides may share the lymphocytic CSF of TBM, and modestly raise CSF protein, but they rarely reduce CSF glucose (with notable exceptions such as mumps meningoencephalitis) or elevate CSF lactate. *Listeria monocytogenes* meningitis results in lymphocytic CSF and should be considered in immunosuppressed individuals, and in the elderly and infants. Cryptococcal meningitis, where CSF parameters may be similar to those in TBM, must be excluded, especially in those with HIV co-infection.

1.3.4 Diagnosis of TBM

1.3.4.1 Conventional diagnostics

The diagnosis of TBM continues to present an enormous challenge to clinicians. Delayed diagnosis of TBM leads to worse outcomes, yet, conventional diagnostic tests are insufficiently sensitive to

consistently detect *M. tuberculosis* in CSF.[54] CSF in TBM is paucibacillary and *M. tuberculosis* bacilli are often present in numbers below the threshold of detection of current tests. Conventional confirmatory tests (identification of *M. tuberculosis* in CSF) include Ziehl-Neelsen (ZN) smear microscopy, *M. tuberculosis* nucleic acid amplification tests (NAAT), and mycobacterial culture. ZN smear microscopy is widely available but is often insensitive unless performed by experienced microscopists using large volumes of CSF that have been optimally processed. Mycobacterial culture allows drug susceptibility testing (DST) if positive, however bacterial growth usually takes at least 2 weeks and results cannot guide important initial anti-TB chemotherapy choice. Two mycobacterial culture methods, MGIT (mycobacteria growth indicator tube) and MODS (microscopic observation drug susceptibility), were shown to be diagnostically equivalent in a study of 156 individuals with suspected TBM in Vietnam.[55]

Elevation of the host enzyme adenosine deaminase (ADA) in CSF may distinguish TBM from other CNS disease. In a recent meta-analysis[56] inclusive of 20 studies, pooled sensitivity and specificity of ADA for TBM diagnosis were 89% and 91% respectively, however variable reference standards and ADA cut-off values for TBM diagnosis, and heterogeneity of included studies, makes widespread clinical application of this testing challenging. Detection of the *M. tuberculosis* cell wall lipoarabinomannan (LAM) antigen in CSF or urine for the diagnosis of TBM is limited by studies showing poor test sensitivity vs. culture, clinical and NAAT standards in predominantly HIV co-infected individuals in Zambia and Uganda.[57,58]

1.3.4.2 Non-confirmatory tests

Non-confirmatory diagnostic tests detect host response to *M. tuberculosis*, rather than *M. tuberculosis* itself. Discovery of metabolomics or proteomic signatures unique to TBM may pave the way for a new diagnostic test. The profiles of 425 metabolites have been characterised in 33 HIV negative Indonesian adults with TBM,[43] and have been shown to differ between TBM and other brain infections in a CSF metabolomic study of 50 individuals with clinical TBM in China.[59] In a study of 47 South African children with suspected TBM, the median levels of 16 host serum protein biomarkers were significantly elevated in those with definite or probable TBM, vs. in those not meeting this TBM diagnosis. An approach focusing on a 3-marker biosignature (adipsin, A β 42, and IL-10) gave 83% and 75% diagnostic sensitivity and specificity respectively.[60] This is a developing field and more data are needed to understand metabolomic and proteomics signatures of TBM.

1.3.4.3 GeneXpert MTB/RIF

Introduced into the diagnostic testing arsenal in 2010, Xpert changed the field of TB diagnostics.[61] Able to return a test result in less than two hours, Xpert is now widely used throughout the world.[62] According to the most up to date WHO data, the estimated percentage of TB cases with multidrug resistance or rifampicin resistance in Vietnam is 4.1% for new cases and 25% for previously treated cases.[63] Neither ZN smear nor MGIT mycobacterial culture can identify rifampicin resistance at diagnosis. Xpert uses a hemi-nested real-time PCR assay to detect and amplify a *M. tuberculosis* specific sequence of the bacterial *ribonucleic acid polymerase (rpoB)* gene.[61] In >95% of cases rifampicin resistance is associated with mutations in the 81 base pair core region of this *rpoB* gene, with this area additionally flanked by *M. tuberculosis* specific DNA sequences; therefore identification of *M. tuberculosis* and detection of rifampicin resistance could be performed in a single test.[64]

However, the paucibacillary nature of CSF in TBM results in a reduced diagnostic performance of Xpert for TBM, vs. sputum testing for pulmonary TB. In recent TBM studies,[37,54,65–67] sensitivity of Xpert ranged from 18-59%, compared with 26-67% for mycobacterial culture.[68] Variable test sensitivities may reflect differences in CSF sampling, processing, testing and differences in host and bacterial genetics, however inconsistent use of the uniform case definition[69] as a reference gold standard for diagnostic test performance analysis also contributes to this variation, and confounds test comparison.

1.3.4.4 The emergence of GeneXpert MTB/RIF Ultra

Xpert represents an ideal diagnostic test for TBM, if test sensitivity could be improved. The new Ultra cartridge aims to improve the sensitivity of TB diagnosis and enhance rifampicin resistance identification, through a larger reaction chamber, plus incorporation of two different multicopy amplification targets (*IS6110* and *IS1081*).[54,70] These cartridge modifications are designed to lower the limit of detection of *M. tuberculosis* colony forming units (CFU); in vitro the lower limit of detection for *M. tuberculosis* decreased to 16 CFU/mL with Ultra, vs. approximately 100 CFU/mL with Xpert.[68,71] Initial small studies[67,72] showed promise for an improved sensitivity of Ultra over Xpert for the diagnosis of TBM. Larger studies comparing Ultra and Xpert for the diagnosis of TBM were required and have now been performed (see chapter 2).

1.3.5 Monitoring of TBM

1.3.5.1 The detection and monitoring of raised intracranial pressure

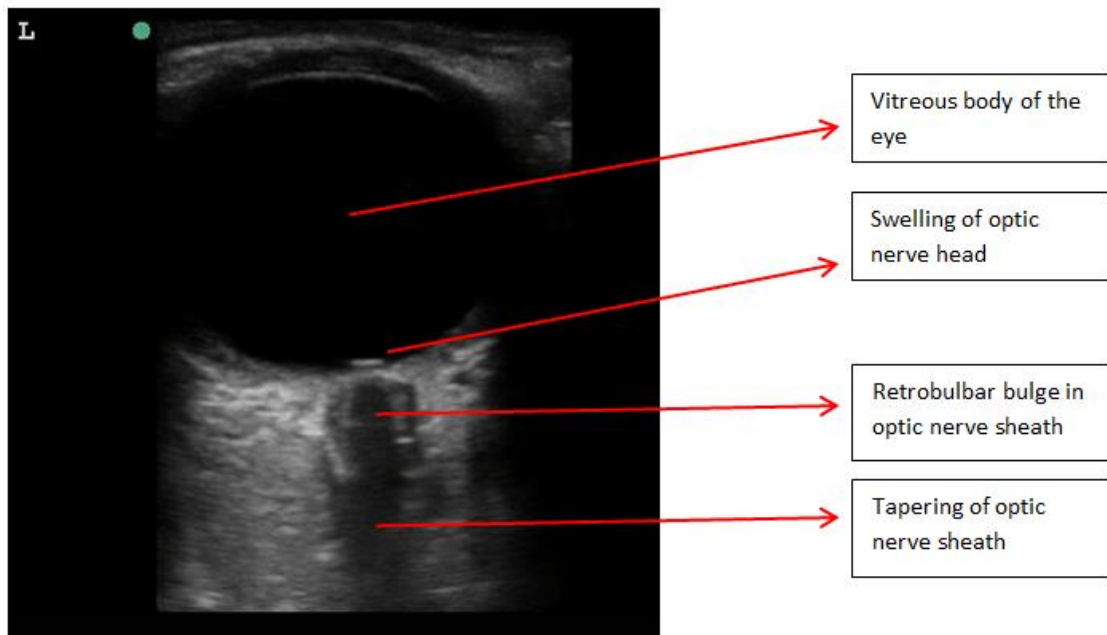
Recognition and management of raised ICP in TBM is important to allow minimisation of intracerebral damage and maintenance of cerebral perfusion. The gold standard for measuring ICP is invasive intracranial monitoring; however, invasive devices and the neurosurgical expertise required to insert them are not available at many centres. Current non-invasive monitoring options are limited. Whilst raised ICP leads to blurring of the margins of the optic disc when viewed by fundoscopy (termed ‘papilloedema’), the development of papilloedema can lag behind elevation of ICP, and there are few data to support a positive correlation between lumbar CSF opening pressure and ICP. Measurement of CSF opening pressure may be performed at the time of lumbar puncture; lumbar puncture is minimally invasive, easy to learn, and equipment is widely available. However, no evidence yet supports opening pressure as a predictor of ICP or clinical outcome in individuals with TBM.

Therefore, alternative non-invasive methods of ICP monitoring have been considered in TBM. Transcranial Doppler ultrasound uses a low frequency (≤ 2 MHz) transducer, placed on the scalp, to scan the basal arteries of the brain and measure cerebral blood flow (CBF) velocity.[73] Transcranial Doppler ultrasound may have a role in identifying raised ICP, however its ability to do this is not without limitations given a decrease in PaCO₂ or an increase in arterial blood pressure may alter CBF independently of changes in ICP.[74]

1.3.5.2 Optic nerve sheath ultrasound

The optic nerve, a part of the central nervous system, is surrounded by a dural sheath that distends when ICP is elevated. This distension can be measured by ultrasound. ONSD ultrasound is a quick, reproducible, reliable, non-invasive and safe way to monitor ICP. An association between ONSD measured by ultrasound and ICP in non-infective brain pathology has now been demonstrated in 3 meta-analyses.[75–77] Data supporting ONSD ultrasound in infective brain pathology are limited however. One small study has demonstrated successful use of ONSD ultrasound in TBM,[78] where a study group of 25 patients with suspected TBM (n=25, mean ONSD 5.81mm) was compared with a healthy control group (where no patients had papilloedema on fundoscopy, n=120) in whom the upper limit of normal for ONSD was 4.37mm.[78] More data are required to support ONSD ultrasound as a tool for detecting and monitoring raised ICP in TBM and as a tool to improve clinical outcomes through improved monitoring. This will be addressed in chapter 4 of this thesis. A distended optic nerve sheath in a Vietnamese adult with TBM is shown in figure 1-1.

Figure 1-1: Distended optic nerve sheath in tuberculous meningitis[79]



An ultrasound image of the left optic nerve sheath in an adult with tuberculous meningitis.

Reference: Donovan J, et al. Lancet Neurol 2019. [http://dx.doi.org/10.1016/S1474-4422\(19\)30154-1](http://dx.doi.org/10.1016/S1474-4422(19)30154-1).

1.3.5.3 Brain imaging

Brain imaging allows identification of the causes (e.g. hydrocephalus, tuberculous masses, cerebral oedema) and consequences (brain shift and ischaemia) of raised ICP. Baseline brain imaging can alter immediate management if hydrocephalus is found, and follow-up brain imaging is recommended for patients with deteriorating symptoms.[9] Therefore brain imaging is a valuable monitoring tool in TBM. However, the best imaging modality, and exactly when to perform imaging, are not known. Brain computed tomography (CT) can identify dilated ventricles, mass effects, infarctions, and inflammatory exudates.[79] Brain magnetic resonance imaging (MRI) provides higher resolution of TBM brain pathology than CT, and has no radiation risks unlike CT, however, it is expensive, time consuming, and may require transfer of a critically ill patient to a hospital with MRI.[80,81] Retrospective data show that both CT and MRI have a role in detecting brain pathology associated with TBM.[82]

1.3.6 Management of TBM

1.3.6.1 Anti-TB chemotherapy

Despite reliance upon drugs that have been available for decades as first line anti-TB chemotherapy, optimum regimens, doses and routes for TBM treatment remain unknown. In severe TBM limited oral administration options, overlapping side effects and interactions with drugs used in critical illness, variable drug CNS penetration, and fewer patients for clinical trials, all contribute to inconsistent, sub-optimal and poorly researched anti-TB chemotherapy regimens. Rifampicin, isoniazid, pyrazinamide, and ethambutol are WHO-recommended first line agents for drug susceptible TB.[83,84] Yet international guidelines[84] are 10 years old, and do not specifically tackle TBM; rather TBM is grouped with other forms of extrapulmonary TB. In Vietnam, rifampicin (10mg/kg/24hrs; maximum 600mg), isoniazid (5mg/kg/24hrs; maximum 300mg), pyrazinamide (25mg/kg/24hrs; maximum 2g) and ethambutol (20mg/kg/24hrs; maximum 1.2g) are given as first line therapies for 2 months, with pyrazinamide then stopped, and rifampicin, isoniazid and ethambutol continued at the same doses to complete 12 months total anti-TB chemotherapy.[41,85]

1.3.6.1.1 Pharmacokinetics

Rifampicin is a critical drug in the treatment of TB, yet its pharmacokinetics, interactions and side effects present challenges for TBM treatment. Rifampicin distributes well throughout the body and freely enters body tissues, except the CNS, where CSF rifampicin levels frequently do not exceed, or only slightly exceed minimum inhibitory concentration (MIC).[15,86–88] Isoniazid is also a critical therapy, despite rising levels of mono-resistance to this drug worldwide. Metabolism of isoniazid includes acetylation by N-acetyltransferase (NAT)-2, with genetic polymorphism in the NAT-2 gene resulting in ‘fast’ or ‘slow’ acetylator phenotypes.[89] Fast acetylation may reduce blood isoniazid levels, thereby reducing efficacy of anti-TB chemotherapy; however, evidence is required to support higher isoniazid dosing in fast acetylators - this is currently the subject of a NAT2 stratified, randomised, parallel group trial in China (NCT03787940). Isoniazid, pyrazinamide and fluoroquinolones penetrate well into the CSF, as do ethionamide and cycloserine.[15,87] Ethambutol and aminoglycosides poorly penetrate the CSF in the absence of meningeal inflammation.[15,86–88] In addition to appropriate therapy choices, prompt treatment and avoidance of therapy interruptions are essential.

1.3.6.1.2 Anti-TB chemotherapy clinical trials

Given the poor CNS penetration of rifampicin, and its importance as a first line therapy, studies of high dose rifampicin dominate the current trial panorama in TBM.[90] Intravenous rifampicin 600mg was associated with reduced mortality vs. rifampicin 450mg orally in an Indonesian

study,[6] but larger studies of intravenous therapy, potentially at higher doses, are required. A randomised trial comparing ‘intensified’ 15mg/kg rifampicin plus 20mg/kg levofloxacin (vs. standard treatment) in 817 Vietnamese adults with TBM did not lead to reduced mortality in the intensified arm,[91] suggesting either anti-TB chemotherapy could not be further optimised in this group, or higher doses or alternative drugs are required. A phase II open-label randomised trial of high dose rifampicin for TBM in adults (RiFT, ISRCTN42218549) is currently ongoing in Uganda, where a 35mg/kg oral rifampicin-containing regimen, with or without an initial two week 20mg/kg intravenous phase, is being compared with a standard oral 10mg/mg/kg rifampicin-containing regimen.[92] The ALTER, (Uganda, NCT04021121), SIMPLE, (Indonesia, NCT03537495) and LASER-TBM trials (South Africa, NCT03927313) will assess pharmacokinetics and safety of 35mg/kg rifampicin in combination with linezolid, a second line drug with potential in TBM. A double-blinded parallel group randomised placebo-controlled phase III trial is due to start recruitment of 500 adults in January 2020, in Indonesia, South Africa and Uganda (HARVEST, ISRCTN15668391). HARVEST will compare anti-TB chemotherapy supplemented with an additional 1.2g rifampicin against fixed-dose combination anti-TB chemotherapy; with assessment of clinical outcomes and safety (including drug induced liver injury [DILI] - the most common drug-associated adverse event in TBM[93]).

1.3.6.2 Drug resistance

The choice of anti-TB chemotherapy regimens must consider the growing threat of drug resistance; TB resistant to first line anti-TB chemotherapy is becoming more common worldwide.[94] Prevalence of multidrug resistance (MDR) in TBM is approximately 4% in Europe[95] and 12% in China.[96] Mono-isoniazid resistance is more common (nine [6%] of 142 cases in the European study[95]) and is associated with worse outcomes, although its effect on treatment response is less than that of MDR, where mortality and morbidity are very high.[97–99] HIV co-infection may impact upon drug resistance and treatment options. In a prospective study of 180 adults with TBM in Vietnam, MDR TBM predicted death, but MDR was also independently associated with HIV co-infection.[98] Whether patient behaviours associate MDR TBM with HIV co-infection, or whether *M. tuberculosis* is more likely to acquire resistance in the context of HIV co-infection, is unknown. In a trial of intensified anti-TB chemotherapy for TBM (15mg/kg rifampicin plus 20mg/kg levofloxacin, vs. standard anti-TB chemotherapy) intensified therapy reduced mortality in isoniazid resistant individuals, but only in those without HIV co-infection.[97] Additionally, isoniazid resistance is not straightforward. Resistance to isoniazid can be high level (usually due to mutations in the *KatG* gene), or low level (due to mutations in the promotor region of *InhA*).[100] Increased

dose isoniazid therapy may be beneficial in low level isoniazid resistance,[101] however evidence is required to support this in TBM. Certainly, early indication of MDR is vital, given this form of TBM is almost always fatal unless second line therapies are administered early.[97] Clinical trial data are needed to guide second line anti-TB chemotherapy in TBM, in addition to treatment data to support any use of newer anti-TB chemotherapies such as delamanid and bedaquiline.

1.3.6.3 Adjunctive corticosteroids

Adjunctive corticosteroids have been shown to reduce mortality from TBM, at least in the short term.[5,28,102] How they confer this clinical benefit is unclear however, although reduction in intracerebral inflammation seems the most plausible mechanism. Glucocorticoids bind to and activate the glucocorticoid receptor of macrophages and other cells, interfering with inflammatory mediator transcription and expression.[103] Additional indirect genomic effects (inhibition of pro-inflammatory transcription factors e.g. activator protein-1), and non-genomic mechanisms, further mediate glucocorticoid anti-inflammatory effects.[104–107]

Additionally, corticosteroids reduce brain oedema, primarily of vasogenic origin, with a limited effect on cytotoxic oedema due to haemorrhage or infarction.[108] Whilst corticosteroids are not beneficial in acute traumatic brain injury (TBI) where they may cause harm,[109,110] they are often used as a rescue therapy in cases of TBM-associated raised ICP, albeit with little evidence to support this use. Corticosteroids are also frequently used to treat neurological IRIS, a common complication of starting ART in TBM, however no controlled trials exist to support corticosteroid use in this condition. Corticosteroids may reduce the size of tuberculomas and control their symptoms however.[111] In a randomised paediatric study corticosteroid therapy reduced mortality, and enhanced the resolution of tuberculomas and basal exudate as seen by brain CT imaging.[112] In a study of Vietnamese adults, there were fewer episodes of MRI-proven hydrocephalus or cerebral infarction at day 60 in individuals receiving corticosteroids, compared with those receiving placebo, although these effects were not statistically significant.[113]

Regardless of the mechanism of benefit, corticosteroid therapy in HIV uninfected individuals with TBM is now recommended, frequently in the form of dexamethasone which is both cheap and widely available. However the optimal corticosteroid preparation, dose, and route of administration, are unknown.[79] Whether beneficial therapeutic effects of corticosteroids extend to HIV co-infected individuals is uncertain. In an HIV co-infected subgroup (n=98) from a randomised trial of adjunctive corticosteroids for TBM in Vietnamese adults, dexamethasone was associated with a non-significant trend towards improved survival.[5] A multicentre randomised controlled trial of

adjunctive corticosteroids for HIV co-infected adults with TBM is currently underway in Vietnam and Indonesia (NCT03092817).[85]

1.3.7 TBM complications and critical illness

1.3.7.1 Supportive care

The management of TBM is complex, extending beyond drug therapies. Supportive care is hugely important; considerations must be given to head of bed elevation, fever control, ventilation strategies, and prevention of pressure damage, ventilator-associated pneumonia, and deep vein thrombosis prevention. Approaches and evidence gaps in the supportive care of individuals with TBM are shown in table 1-2.

Table 1-2: Approaches and evidence gaps in the supportive care of individuals with tuberculous meningitis[79]

Infection control and reducing potential in-hospital <i>M. tuberculosis</i> transmission	<ul style="list-style-type: none"> • Respiratory isolation may be required in those with concomitant pulmonary TB. • Respiratory isolation in critical care is challenging when high patient visibility is required. • The effect of endotracheal intubation and a closed ventilation circuit on TB transmission is uncertain, given periodic breaks to this closed circuit to allow suction may induce coughing.
Head-of-bed elevation to reduce ICP	<ul style="list-style-type: none"> • Elevating the head end of a bed aids venous drainage and shifts CSF extracranially, but may also lower mean arterial pressure • The effect of head-of-bed elevation in TBM has not been studied. An optimal elevation is unknown.
Maintaining normal glucose concentrations	<ul style="list-style-type: none"> • Hyperglycaemia and hypoglycaemia adversely affect the brain during critical illness, however optimal glucose control is not known.[114]
Treating anaemia to ensure optimal tissue oxygenation	<ul style="list-style-type: none"> • Haemoglobin is important for optimal oxygen delivery in patients at risk of ischaemia, and optimal transfusion thresholds have not been determined in TBM.
Controlling and reducing fever	<ul style="list-style-type: none"> • It is not known if treating fever in TBM improves outcomes. • No trials describe therapeutic hypothermia in TBM.

Deep vein thrombosis prevention	<ul style="list-style-type: none"> • The role of deep vein thrombosis prophylaxis in critically ill individuals with TBM, where corticosteroids and aspirin may add to gastrointestinal bleeding risk, has not been studied. • The effect of head-of-bed elevation or general patient positioning in TBM on deep vein thrombosis has not been studied.
Protecting pressure areas	<ul style="list-style-type: none"> • The effect of head-of-bed elevation or general patient positioning in TBM on pressure area damage has not been studied.
Ventilator-associated pneumonia prevention	<ul style="list-style-type: none"> • Mechanical ventilation is common in critical care of TBM (70% in a study by Cantier et al).[115] Almost one quarter of mechanically ventilated TBM patients developed ventilator-associated pneumonia in a study by Misra et al.[116] • Ventilator-associated pneumonia prevention strategies uncertain in TBM
Optimising nutrition	<ul style="list-style-type: none"> • TB is a catabolic illness yet no nutritional guideline exists specifically for TBM.

CSF=cerebrospinal fluid. ICP=intracranial; pressure. TB=tuberculosis. TBM=tuberculous meningitis. Reference: Donovan J, et al. Lancet Neurol 2019. [http://dx.doi.org/10.1016/S1474-4422\(19\)30154-1](http://dx.doi.org/10.1016/S1474-4422(19)30154-1).

1.3.7.2 Polypharmacy

Critical illness may be caused by, or exacerbated by, anti-TB chemotherapy or other drugs. Important side effects, and interactions of drugs used in the treatment of critically ill individuals with TBM are shown in table 1-3. DILI may manifest as vomiting, abdominal pain, jaundice, and elevated liver function tests. Any drug reactions affecting neurological status may interfere with patient monitoring and assessment of GCS. Isoniazid and fluoroquinolones,[91,117] both used in the treatment of TB, increase seizure risk. A study of patients with TBM or tuberculoma experiencing seizures found that co-administration of isoniazid and phenytoin resulted in significantly higher serum phenytoin levels in slow acetylators compared with fast acetylators.[118] Yet, acetylator status (NAT-2 genotype) is infrequently tested. Given the interaction of anti-TB chemotherapy and anti-convulsants, the cytochrome (CY) P450 inducing effects of rifampicin and multiple anti-convulsants, the CYP450 inhibiting effect of isoniazid, and the role of benzodiazepines as a CYP3A4 substrate with a narrow therapeutic window, anti-convulsants must

be used with caution in critically ill individuals with TBM. Sodium, potassium, and glucose abnormalities may result from TBM treatment strategies.

Table 1-3: Drugs commonly used during management of tuberculous meningitis[79]

Drug	Therapeutic role in TBM	Primary adverse drug effect/side-effect	Additional drugs affected	Additional adverse/side effects
Rifampicin	First line ATT	- Inducer of cytochrome 3A4 enzyme[119]	- Reduction in serum levels of anti-retroviral therapy including NNRTIs and PIs[119] - Benzodiazepines; substrates for cytochrome 3A4[120]	DILI, hypersensitivity including SJS, renal failure, adrenal insufficiency, haemolysis, cytopenia[119]
Isoniazid	First line ATT	- Inhibitor of cytochrome 3A4[121]	- Phenytoin; higher serum phenytoin levels in slow acetylators[118] - Benzodiazepines; substrates for cytochrome 3A4[120]	DILI, neuropathy, seizures, psychiatric disorders, TEN, SJS, pancreatitis, haemolysis, cytopenia[121]
Pyrazinamide	First line ATT			DILI, hypersensitivity including urticarial
Ethambutol	First line ATT	Ocular toxicity difficult to detect in comatose patient		Ocular toxicity, hypersensitivity including SJS, thrombocytopenia, leucopenia, renal failure[122]
Quinolones (e.g.	ATT (second	- Lowers seizure		DILI, seizures,

levofloxacin, moxifloxacin, gatifloxacin)	line agents)	threshold, demonstrated in higher dose rifampicin plus levofloxacin arm of TBM trial[91]		psychiatric disorders, QT prolongation, TEN, SJS, haemolysis, cytopenia, renal failure, tendinitis[123]
Corticosteroids (e.g. dexamethasone)	Adjunctive anti-inflammatory drug	Gastrointestinal bleeding risk higher in critically ill patients	Aspirin; overlapping side effect profile	Adrenal insufficiency on discontinuation, gastrointestinal bleeding, psychosis, infections
Aspirin	Anti-platelet / anti-thrombotic agent	Gastrointestinal bleeding risk higher in critically ill patients	Corticosteroids; overlapping side effect profile	Gastrointestinal bleeding, hypersensitivity reactions
Acetazolamide	- Reduction of CSF production[124]	May cause hyponatraemia in a condition where hyponatraemia is common and hazardous	- Overlapping treatment effect profile with fludrocortisone - Increased metabolic acidosis and neurological effects with concomitant aspirin, interactions with anti-convulsants[125]	- Electrolyte imbalance, renal failure - Extensive side effects and drug interactions, covered elsewhere[126]

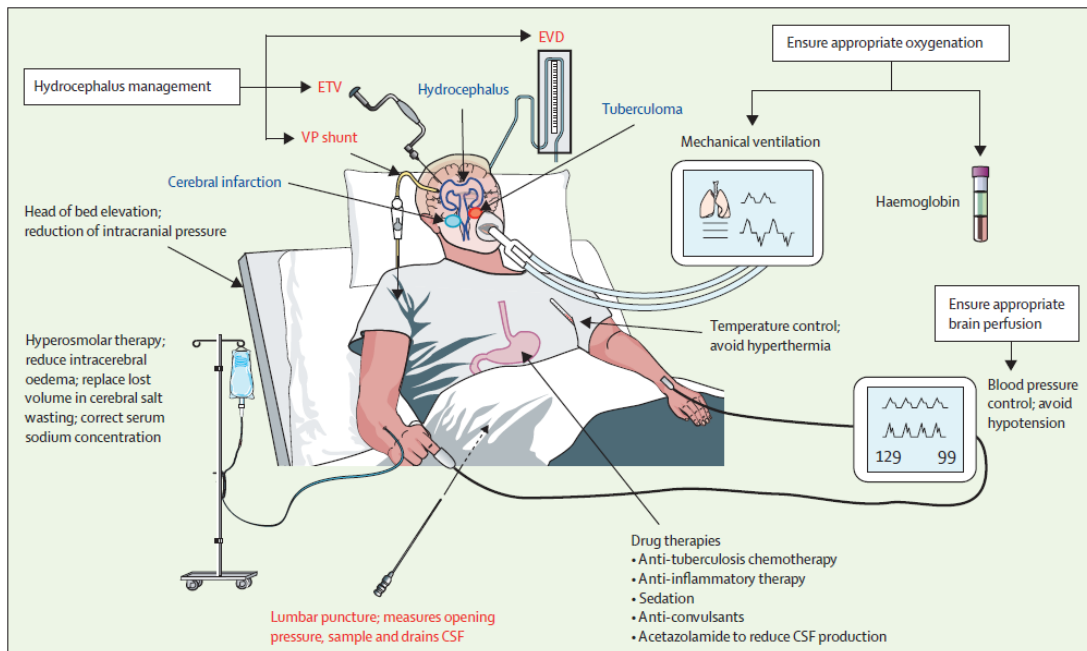
ATT=anti-tuberculosis chemotherapy. ART=anti-retroviral therapy. CSF=cerebrospinal fluid. DILI=drug induced liver injury. NNRTIs=non-nucleoside reverse transcriptase inhibitors. PIs=protease inhibitors. SJS=Stevens-Johnson syndrome. TBM=tuberculous meningitis. TEN=toxic

epidermal necrolysis. Reference: Donovan J, et al. Lancet Neurol 2019. [http://dx.doi.org/10.1016/S1474-4422\(19\)30154-1](http://dx.doi.org/10.1016/S1474-4422(19)30154-1).

1.3.7.3 Neurological complications

Numerous disease complications reduce consciousness, often through raised ICP which, as discussed, may be challenging to detect. Hydrocephalus, cerebral oedema, inflammatory complications such as paradoxical reactions and neurological IRIS, and cerebral infarction, all may contribute to raised ICP in varying degrees.[78] Whether targeted ICP-guided therapies upon detection of raised ICP improves outcomes in TBM is an area for future research. Identification of individuals who will benefit from neurosurgical management of raised ICP is difficult. Reduction and control of ICP, and maintenance of brain perfusion are critical in TBM, as illustrated in figure 1-2. Below, the most common neurological complications of TBM are briefly discussed.

Figure 1-2: Potential strategies for the management of intracranial pressure and maintenance of brain perfusion in critically ill individuals with tuberculous meningitis[79]

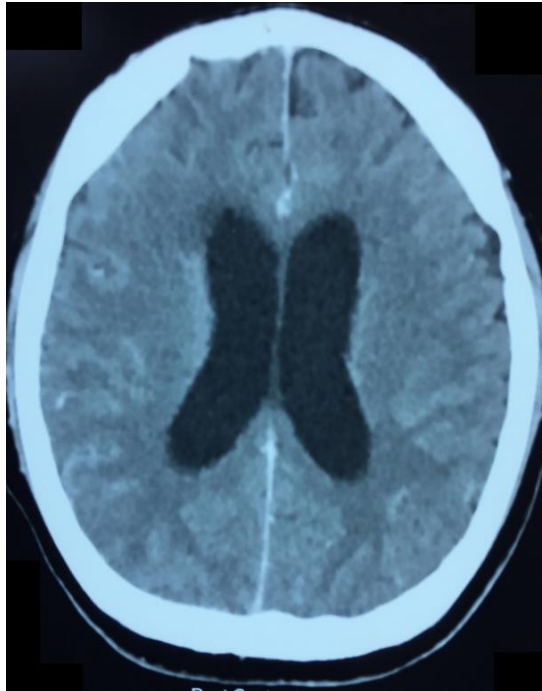


Little evidence exists to guide these strategies in TBM; however, this figure illustrates potential approaches. Surgical interventions are labelled in red, disease processes are labelled in blue, and other interventions labelled in black. ETV=endoscopic third ventriculostomy. EVD=external ventricular drain. TBM=tuberculous meningitis. VP=ventriculoperitoneal. Reference: Donovan J, et al. Lancet Neurol 2019. [http://dx.doi.org/10.1016/S1474-4422\(19\)30154-1](http://dx.doi.org/10.1016/S1474-4422(19)30154-1).

1.3.7.3.1 Hydrocephalus

Hydrocephalus occurs when there is impairment of the passage of CSF from its point of production to its point of absorption (figure 1-3).[127] Hydrocephalus occurs in approximately 70% of TBM overall,[128] with higher rates reported in children; 80-86%,[129–133] than in series predominantly of adults; 43-48%.[134,135] Hydrocephalus is rare in early TBM, but almost always present once neck stiffness is present and loss of consciousness has occurred.[15] TBM-associated hydrocephalus is communicating in approximately 80% of cases, due to basal cistern block,[15,136,137] and medical therapy (acetazolamide plus furosemide) may be effective in these cases.[138] Obstruction of CSF flow at the brain aqueduct, or more commonly at the fourth ventricle foramina, results in non-communicating hydrocephalus.[15,139] Cerebral aqueduct obstruction occurs via constriction by exudate surrounding the upper brainstem, or an intraluminal tuberculoma, whereas the fourth ventricle outlet is obstructed by exudate.[128,139] Distinction between communicating and non-communicating hydrocephalus is vital, yet this is not reliably identified using conventional brain CT imaging or MRI.[140] Lumbar CSF drainage in non-communicating hydrocephalus can widen the pressure differential across the two (or more) separated CSF compartments, resulting in cerebral tonsillar herniation.

Figure 1-3: Hydrocephalus in tuberculous meningitis



CT imaging showing distended lateral ventricles of the brain (central black spaces) due to raised intraventricular pressure; this is hydrocephalus. CT=computed tomography.

1.3.7.3.2 Cerebral infarction

Cerebral infarcts are the main cause of irreversible brain damage in TBM,[141] occurring in 8-67% of patients.[141–147] The pathophysiology of cerebral infarction may depend upon the stage of TBM, with vasospasm more common than vasculitis in early disease.[141] In later disease vessel pathology likely occurs through a combination of infiltrative, proliferative and necrotising processes.[141,147] The role of thrombosis is unclear.[141] CT imaging in TBM has shown basal cistern exudate enveloping arteries, leading to arteritis, and endarteritis infiltration, particularly of small perforating arteries of the circle of Willis. Less frequently, occlusion of the middle cerebral artery or anterior cerebral artery cause major infarction.[148] Infarcts are frequently multiple.[144] Clinical presentation of cerebral infarction varies, however hemiplegia appears to be the most common presenting sign.[15,141] Long term neurological disability is common, prolonging hospital stay and time in bed, and increasing the risks of non-neurological complications such as ventilator associated pneumonia and pressure area damage. No adjunctive treatment has consistently reduced stroke or hemiplegia in TBM.[15]

1.3.7.3.3 Tuberculomas

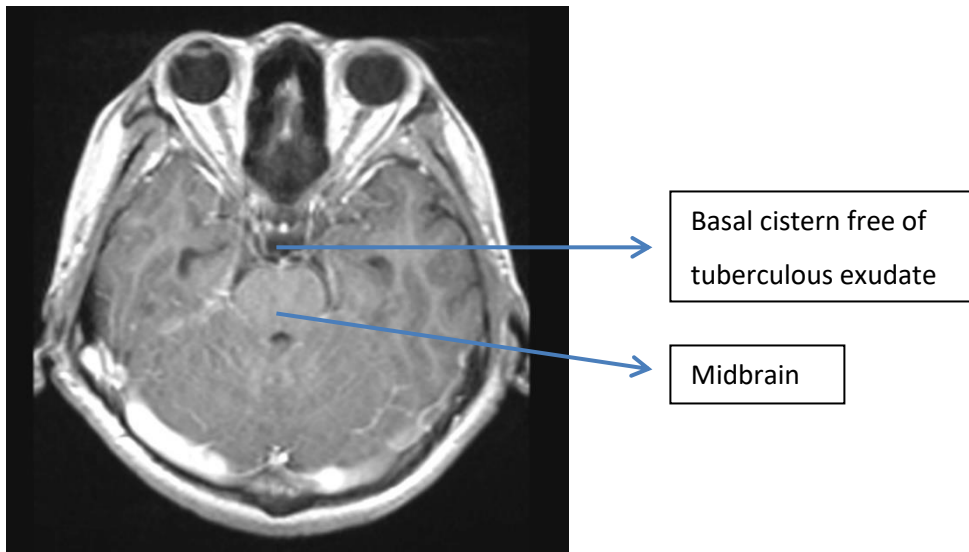
Tuberculomas may develop with or without TBM, and may develop or enlarge during appropriate anti-TB chemotherapy. If a tuberculous granuloma (Rich focus) does not rupture but instead continue to grow, a tuberculoma can result. Additionally tuberculomas may develop from resolving granulomatous inflammation weeks after commencing anti-TB chemotherapy, despite anti-TB chemotherapy being appropriate.[15] Studies of adults and children have reported an incidence of tuberculomas on imaging at 2-42%.[131,134,135] In a study of 43 adults with TBM randomised to dexamethasone or placebo in Vietnam, receiving dexamethasone did not affect the site or number of tuberculomas, nor the proportion of patients affected by a tuberculoma at day 60 or day 270 of treatment.[113] In an adult study, brain MRI revealed tuberculomas in 64% (14/22) patients at baseline, and 74% (20/27) patients at day 60,[113] whilst in a paediatric study brain MRI (average 26 days post-admission) found tuberculomas in 59% (23/39) patients, compared with only 7% (3/44) patients using CT on admission in the same group.[149] Tuberculomas may exert mass effect upon brain tissue, or compression of the cerebral ventricles leading to a non-communicating hydrocephalus.

1.3.7.3.4 Paradoxical reactions

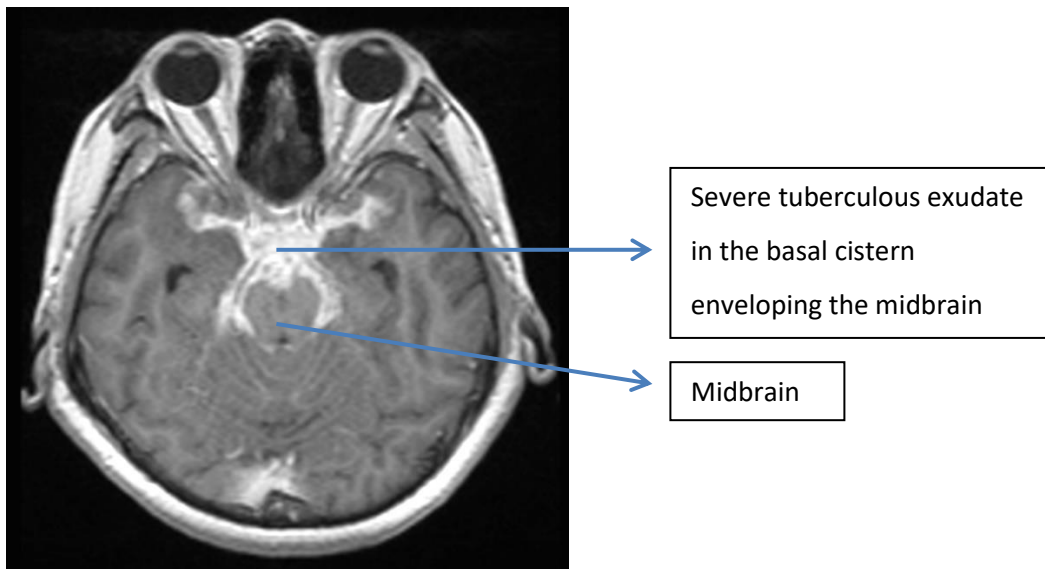
Paradoxical reactions manifest as worsening of an existing lesion or the appearance of new lesions, despite an initial response to treatment. Although most commonly considered an exuberant inflammatory response to dead or dying bacteria, their pathogenesis is poorly understood.[79,150] In a study of patients in India (mean age 30 years), 31% (44/141) patients developed a paradoxical reaction.[151] The timing of onset of paradoxical reactions is highly variable however onset is usually after 2-4 months of anti-TB chemotherapy.[152] In the context of TBM paradoxical reactions present as fever, headache, altered sensation or vision, or seizures, in addition to a worsening of CSF cell count or protein.[151] Tuberculomas are the most common paradoxical reaction in HIV uninfected TBM.[152] Basal cistern exudate may develop after commencement of anti-TB chemotherapy; this may be seen on follow up brain imaging, despite normal baseline imaging (figure 1-4).

Figure 1-4: Basal cistern exudate of tuberculous meningitis

Panel A:



Panel B:



Panel A: Baseline MRI brain (transverse view) from a 22 year old male with tuberculous meningitis. Clinical presentation was with a 3-week history of fever, headache and neck stiffness, with no neurological deficit. Panel B: MRI brain (transverse view) taken after same patient readmitted with an acutely reduced level of consciousness and bilateral cranial nerve palsies, 3 weeks after first imaging. Repeat imaging showed severe basal meningeal exudate secondary to tuberculous meningitis. MRI=magnetic resonance imaging

In HIV co-infected individuals who have newly commenced ART, paradoxical reactions are usually attributed to IRIS. Imaging in TBM IRIS can reveal new or enlarging tuberculomas with or without meningitis, infarcts, hydrocephalus and radiculomyopathy, or less commonly optochiasmatic or spinal arachnoiditis.[151,153] A case series of neurological IRIS in South African patients with TBM suggested IRIS has considerable short term morbidity but reasonable long term outcomes.[153]

The optimal timing for commencing ART in TBM, to allow immunological recovery without IRIS, is unknown. A study of 34 ART-naïve HIV co-infected patients with TBM found the occurrence of neurological IRIS (47% [16/34] patients) to be associated with higher CSF neutrophil counts and *M. tuberculosis* culture positivity at diagnosis. All patients received prednisolone 1.5mg/kg/day alongside anti-TB chemotherapy, and all patients commenced ART 2 weeks after starting anti-TB chemotherapy.[23] A randomised double-blind placebo-controlled trial compared immediate ART (within 7 days of starting anti-TB treatment) with deferred ART (2 months after starting anti-TB chemotherapy) in 253 Vietnamese adults with TBM;[154] in the immediate arm there was no reduction in mortality and this group experienced more grade 4 adverse events. In clinical practice in Vietnam, ART may be commenced 8 weeks after anti-TB chemotherapy, however, in individuals with normal baseline brain imaging and low CSF neutrophil counts, ART may be started earlier.

1.3.7.3.6 Spinal disease

Spinal disease is under recognised in TBM, yet contributes significantly to morbidity. Most commonly due to tuberculous arachnoiditis, spinal involvement is often only revealed paradoxically after commencing anti-TB chemotherapy, although high CSF protein may indicate a higher risk of development.[155] Neurological manifestations depend upon the affected level, however lumbosacral disease is most common.[155] It may be challenging to clinically recognise spinal disease in individuals with severe TBM disease in whom history is unavailable, and neurological examination is frequently unreliable. Reduced mobility resulting from spinal disease increases bed-time and secondary complications of this such as damage to pressure areas. Urinary retention is often the first sign presenting to the clinician. Outcomes from spinal disease are usually poor.

1.3.7.4 Hyponatraemia

Hyponatraemia is a common electrolyte imbalance in individuals with TBM. Cerebral salt wasting (CSW) and the syndrome of inappropriate antidiuretic hormone secretion (SIADH) are considered the likely causes, however the pathogenesis of these conditions in TBM is not well understood. CSW may be mediated via hormonal mechanisms (likely via atrial natriuretic peptide [ANP],

released from atrial muscle after atrial stretch), or via direct effects on neural connections to the kidneys, where interruption of sympathetic stimulation leads to increased renal blood flow and natriuresis.[156] Whilst ANP containing neurons exist in the brain, low brain ANP levels make it unlikely that production at this site is the mechanism of CSW.[156] ANP and brain natriuretic peptide (BNP) act in the brain to decrease salt appetite, water intake and corticotrophin release, and act in the kidney to decrease aldosterone and renin, resulting in renal sodium loss.[157] Conversely, SIADH occurs when anti-diuretic hormone is secreted independently of the body's need to conserve water.[158]

Distinguishing between CSW and SIADH is hugely challenging, given the need for extracellular fluid assessment. Determining the exact cause of hyponatraemia in TBM may indeed have limited clinical value; with the exception of avoiding fluid restriction in a high urinary volume, high urinary sodium, scenario. In this scenario haemodynamic instability rapidly results and intravenous fluid becomes mandatory. CSW and SIADH may in fact overlap or occur in sequence, confounding assessment further.

Whether to correct hyponatraemia in the absence of hypovolaemia is uncertain, and no trials of sodium-containing fluids, sodium concentrations, or fluid durations, have been performed to date in TBM. A randomised controlled trial (RCT) compared intravenous and oral sodium supplementation with or without fludrocortisone (0.1-0.4 mg/day) in the treatment of 37 Indian adults with hyponatraemia (<135 mEq/L) caused by CSW associated with TBM.[159] Fludrocortisone (combined with intravenous and oral salt supplementation) was significantly associated with faster correction of plasma sodium than intravenous and oral salt supplementation alone (4 days vs. 15 days), but did not influence mortality or disability at 6 months. Fludrocortisone was associated with severe hypokalaemia and hypertension in two patients with hyponatraemia necessitating its discontinuation.[79] In this thesis (chapter 5) I will perform a large longitudinal (first 30 days) descriptive analysis of hyponatraemia in TBM.

1.3.8 Summary

TBM carries an unacceptably high mortality and morbidity. To achieve progress in the field and improve patient outcomes, numerous challenges must be overcome and research gaps closed. True global incidence of TBM is uncertain, and the disease may be hard to recognise for the non-specialist clinician. Current diagnostic tools for identifying *M. tuberculosis* in CSF are insufficiently sensitive, resulting in delayed treatments, which only worsens prognosis further. TBM treatment courses are lengthy with drug resistance a growing problem. Few clinical trial data are available to

guide best treatments. Numerous disease complications exist, and are challenging to detect, monitor, and manage.

Yet the TBM research field is not remaining still. A recently formed TBM Research Consortium allows collaboration and discussion. Eleven TBM clinical trials have started or are due to start imminently, including two in paediatrics where data are especially sparse. More understanding is emerging regarding the complex TBM pathophysiology, and bacterial-host interaction. A growing ability to understand and quantify -omics data may lead to advances in diagnostics, advances in prognostic biomarkers, and may identify new therapeutic targets. Increasing global recognition of TBM, through development of specific TBM guidelines, involvement of TBM at international TB conferences, and more TBM-directed funding, is vital to raise the profile of TBM and continue the forward direction of research in this field.

1.4 Publications related to this chapter

Aspects of this introductory chapter appear in the following published review article:

1. The neurocritical care of tuberculous meningitis

Joseph Donovan, Anthony Figaji, Darma Imran, Nguyen Hoan Phu, Ursula Rohlwink, Guy E. Thwaites

The Lancet Neurology 2019 Aug;18(8):771-783

Chapter 2

A randomised comparison of GeneXpert MTB/RIF and GeneXpert MTB/RIF Ultra for the diagnosis of tuberculous meningitis

2.1 Introduction

2.1.1 Tuberculous meningitis: why do we need a new diagnostic test?

TBM is the most severe form of TB, resulting in death or disability in approximately half of those it affects.[15] Delayed diagnosis and treatment are strongly linked to poor outcomes. Yet diagnosis of TBM is challenging and has remained so for many years. The indolent meningitic presentation of TBM mimics that caused by other pathogens; as such, a diagnosis of TBM may not be considered by the treating clinician. A consistent clinical history, CSF parameters, and chest X-ray (miliary or pulmonary TB) may increase the clinician's suspicion of TBM; however, confirming a diagnosis of TBM rests upon the detection of acid-fast bacilli (AFB) in CSF.

Unfortunately, the low number of *M. tuberculosis* bacilli in CSF makes their identification difficult. Current diagnostic tests lack sufficient accuracy to exclude TBM on the basis of a negative result. Current confirmatory diagnostic tests for TBM include smear microscopy (for example the ZN method of staining AFBs), mycobacterial culture, and molecular tests such as Xpert. Each of these three testing methods is currently performed at our research unit; OUCRU based at HTD in Ho Chi Minh City. Each of these three testing methods has limitations, which need consideration when these tests are used as a reference standard for new diagnostic tests. In this thesis chapter I will describe ZN smear microscopy and mycobacterial culture (confirmatory tests for the study described in this chapter), in addition to molecular assay testing (including Xpert). Additional diagnostic tests for TBM, confirmatory and non-confirmatory, are further discussed in chapter 1.

2.1.2 Current confirmatory diagnosis tests

2.1.2.1 Ziehl-Neelsen smear microscopy

CSF ZN smear microscopy is the most basic test for TBM, requiring readily available laboratory equipment and an appropriate space to perform the test. A large volume of CSF (~10mls) [9] should first be centrifuged, enabling the concentration of AFBs into a pellet. Resuspended CSF pellet is pipetted onto a cleaned and labelled microscope slide. After drying, the sample is flooded with carbol-fuchsin, and then heated to drive the stain into the mycolic acid wall of *M. tuberculosis*, if it

is present. After decolourisation with acid-alcohol, a counterstain such as methylene blue is added. The slide is dried, and then read under a 100x lens using oil immersion.

Whilst these are the general principles of CSF ZN smear microscopy, methods are variable between different centres, and diagnostic sensitivity globally is often poor.[65] Pre-centrifugation CSF volume, duration of microscopy, and microscopist experience are all important for improving *M. tuberculosis* identification.[65,160] Killed mycobacteria will also stain positive; a confounding factor for analysis of repeat clinical samples after initiation of anti-TB chemotherapy. Additionally, AFB include non-tuberculous mycobacteria, and non-mycobacterial bacteria such as *Nocardia species*; however, these are unlikely to be present in CSF, where AFBs invariably represent TBM.

2.1.2.2 Mycobacterial culture

Culture of *M. tuberculosis* from CSF offers proof of the organism and fully confirms TBM. Given *M. tuberculosis* grows faster in liquid media than solid media,[161] liquid mycobacterial culture methods such as MGIT and MODS are favoured in TBM (vs. solid culture). In MGIT culture (for example the BACTEC MGIT 960 system), mycobacteria are cultured in media together with mycobacterial growth supplements and antibiotics; *M. tuberculosis* growth is identified via detection of fluorescence from an oxygen sensitive compound in the tube.[162] With MODS, the characteristic cord formation of *M. tuberculosis* is viewed in liquid culture by regular microscopy.[161] MGIT and MODS were shown to be equivalent for the diagnosis of TBM in a study of 156 individuals in Vietnam.[55] Although cheaper than MGIT, MODS is time consuming, and may increase risk of exposure to aerosolised mycobacteria; however, this risk is uncertain with TBM where CSF has lower bacillary load than sputum.

Mycobacterial culture sensitivity rarely exceeds 60%, and is often lower.[37,65,67] When *M. tuberculosis* does grow in culture media, DST can be performed, and anti-TB chemotherapy can be modified if resistance is found. Mycobacterial culture from CSF often takes many weeks and therefore cannot guide initial treatment. Like smear microscopy, optimisation of sample (large volume, centrifugation) is critical to increase the chances of *M. tuberculosis* growth and a positive result.

2.1.2.3 GeneXpert MTB/RIF

According to the most up to date WHO data, the estimated percentage of TB cases with MDR or rifampicin resistance in Vietnam is 4.1% for new cases and 25% for previously treated cases.[63] Given neither ZN smear microscopy nor mycobacterial culture can identify rifampicin resistance at diagnosis, development of a new diagnostic test was essential. Xpert offered a breakthrough in TB

diagnostics; a rapid high sensitivity NAAT with additional rifampicin susceptibility testing. Xpert uses a hemi-nested real-time PCR assay to detect and amplify a *M. tuberculosis* specific sequence of the bacterial *rpoB* gene.[61] In >95% of cases rifampicin resistance is associated with mutations in the 81 base pair core region of the *rpoB* gene, with this area additionally flanked by *M. tuberculosis* specific DNA sequences; therefore identification of *M. tuberculosis* and detection of rifampicin resistance can be performed in a single test.[64] Xpert results are available in less than two hours,[61] and testing systems allow multiple samples to be tested (each within its own cartridge) simultaneously.

Xpert is now widely distributed throughout the world; over 7500 Xpert systems have been deployed globally, including over 3500 installed in 110 high burden developing countries.[62] As with smear microscopy and mycobacterial culture, patient factors, clinical sampling, and sample processing can all influence Xpert sensitivity; HIV co-infection,[33] large CSF volume, CSF centrifugation, and the addition of a vortexing step after addition of sample reagents,[37] all increase the chances of a positive test result.

2.1.3 Evidence supporting GeneXpert MTB/RIF for tuberculosis testing

2.1.3.1 Pulmonary tuberculosis

Xpert can be used to identify *M. tuberculosis* in a variety of clinical samples. A Cochrane review of the performance of Xpert for the detection of *M. tuberculosis* in sputum found Xpert to have high sensitivity and specificity for diagnosing pulmonary TB (pooled sensitivity 89%, pooled specificity 99%).[163] In individuals with pulmonary TB and HIV co-infection pooled sensitivity reduced to 79%, consistent with a reduced sputum bacillary load in HIV co-infection.

2.1.3.2 Tuberculous meningitis

The diagnostic performance of Xpert for *M. tuberculosis* identification in CSF unfortunately does not match the same high performance of Xpert with sputum samples, likely due to the paucibacillary nature of CSF in TBM. A South African study of 204 individuals (87% HIV co-infected) with suspected TBM found Xpert to be 67% sensitive at detecting *M. tuberculosis* in CSF (pooled centrifuged and uncentrifuged samples) vs. a liquid culture or Amplicor PCR positive reference standard.[164] When the diagnostic sensitivity of Xpert for the detection of *M. tuberculosis* in the same CSF samples was compared to a liquid culture or Amplicor PCR positive reference standard, plus the meeting of criteria for probable or definite TBM (defined by the uniform case definition for TBM[69]), Xpert sensitivity reduced to just 36% for TBM diagnosis.

These differing sensitivities, 67% vs. 36%, neatly demonstrates the issue of reference standard use when comparing diagnostic tests for TBM, a disease for which there is no gold standard for test comparison.

A study of Vietnamese adults with TBM showed Xpert sensitivity to be 59.3% (108/182; 95% CI 51.8-66.5%) compared to a clinical diagnosis of TBM (definite, probable, and possible), with test specificity 99.5% (95% CI 97.2-100%).[37] The addition of a vortexing step to the Xpert procedure (after addition of sample reagent to resuspended CSF, in order to facilitate cell breakdown and release of intracellular *M. tuberculosis* DNA) part way through data collection in this study, improved Xpert sensitivity for TBM diagnosis; without vortexing: 50.0% (13/26 [95% CI 29.9-70.1%]) vs. with vortexing: 60.9% (95/156 [95% CI 52.8-68.6%]). This study [37] also demonstrated the impact of CSF volume and HIV co-infection on Xpert sensitivity. Xpert sensitivity was assessed by CSF volume tested, and by HIV co-infection status. Using the same reference standard of definite probable and possible TBM, sensitivities of Xpert by CSF volume tested were; 51.7% (95% CI 32.5-70.6%) for low-volume (≤ 2 ml) samples, 61.5% (95% CI 44.2-73.0%) for medium-volume (2.1-5.0ml) samples, and 59.2% (95% CI 44.2-73.0%) for high-volume (> 5 ml) samples.[37] In participants with HIV co-infection, Xpert sensitivity was 78.8% (95% CI 77.6-79.7%), and in HIV-uninfected participants sensitivity was 47.9% (95% CI 47.0-48.7%).

Bahr et al described the performance of Xpert for the identification of *M. tuberculosis* in CSF samples of patients with meningitis in whom cryptococcal meningitis had been excluded.[165] Xpert was compared against microbiologically proven TBM using CSF that was non-centrifuged (Xpert sensitivity 28%) and centrifuged with subsequent resuspension (Xpert sensitivity 72%).[165] In this study, when Xpert testing of centrifuged CSF was compared with definite or probable TBM (using the uniform case definition[69]), sensitivity of Xpert for *M. tuberculosis* detection was 65%, with a specificity of 100%. [165] Sensitivity reduced to just 20% when Xpert was compared with definite, probable and possible TBM.[165]

2.1.3.2.1 Meta-analyses

A meta-analysis (13 studies, 839 samples) of Xpert performance for TBM diagnosis compared vs. mycobacterial culture as a reference standard found a pooled sensitivity of Xpert of 80.5% (95% CI 59.0-92.2%), and a pooled specificity of 97.8% (95% CI 95.2-99.0%).[166] In this study, when Xpert was compared against clinical reference standards (inclusive of presenting signs, symptoms, biochemical and microbiological tests; defined by the individual studies) the pooled sensitivity of Xpert for TBM diagnosis was 62.8% (95% CI 47.7-75.8%), and the pooled specificity was 98.8%

(95% CI 95.7-100%) (5 studies, 711 samples). A WHO meta-analysis of studies published between 2007 and 2012 inclusive, found 16 studies (709 samples) where CSF tested by Xpert was compared against CSF culture as a reference standard.[167] In this analysis pooled sensitivity was 79.5% (95% CI 62.0-90.2%) and the pooled specificity was 98.6% (95% CI 95.8-99.6%).[167]

2.1.4 Alternative molecular tests to Xpert platforms

Whilst Xpert tests are the dominant molecular assays worldwide for TB and TBM diagnosis, alternative diagnostics are in development and testing. Loop-mediated isothermal amplification (LAMP) is a unique isothermal (constant temperature) test-tube based loop DNA amplification technique, which has been applied to *M. tuberculosis* (TB-LAMP; Eiken Chemical Company Ltd, Tokyo, Japan), and is now recommended by the WHO as an alternative to sputum smear microscopy for pulmonary TB diagnosis.[168] LAMP utilises specially designed primers to attach to target DNA, resulting in the production of loop-DNA structures which can continue to amplify under constant temperature.[169] The isothermal approach reduces cost, the test is quick (< 1 hour), and the colour reaction within the test tube can be read by the naked eye. Critically, LAMP cannot identify rifampicin resistance. In a 2018 meta-analysis of LAMP performance for extrapulmonary TB diagnosis, pooled LAMP sensitivity and specificity were 76% and 99%, respectively, for the identification of *M. tuberculosis* in CSF (4 studies, variable composite reference standards).[170]

The new molecular assays Truenat MTB and Truenat MTB Plus (Truenat) (Molbio Diagnostics, Goa, India) are semi-automated chip-based PCR tests that use 2 individual devices (1: sample preparation and DNA extraction, 2: PCR) to identify *M. tuberculosis* and then optionally proceed to identification of rifampicin resistance if required.[171] Preliminary data suggest Truenat tests have comparable diagnostic accuracy with Xpert tests when testing sputum of suspected pulmonary TB.[172] There are no published studies describing Truenat for the diagnosis of TBM.

2.1.5 Reference standards for TBM diagnostics

2.1.5.1 Inconsistent use of reference standards

Whilst meta-analyses show impressive sensitivity for *M. tuberculosis* identification in CSF by molecular methods, against a standard of mycobacterial culture,[166,167], diagnostic sensitivity falls when a reference standard more representative of true TBM cases is used. A reference standard of positive mycobacterial culture does not reflect all cases of TBM, many of which are culture negative.

Inconsistency in reference standard use for TBM diagnostic tests leads to confusion regarding test sensitivity, and limits comparison between studies. Diagnostic test comparison against a reference standard that includes only ZN smear or mycobacterial culture positive individuals likely selects a reference standard containing higher bacillary loads, resulting in a misleading conclusion of higher test sensitivity, as the test under investigation (e.g. Xpert) now has a greater chance of identifying *M. tuberculosis* bacilli. An ideal reference standard should identify and include all patients with TBM.

2.1.5.2 The uniform case definition for tuberculous meningitis

The uniform case definition for TBM[69] offers a system for the standardisation of TBM research. This case definition incorporates clinical data, CSF parameters, brain imaging, evidence of extraneural TB, and positive diagnostic tests, to categorise suspected TBM cases into 1 of 4 diagnostic groups; definite, probable, possible, and not-TBM. However, in the absence of a ‘gold standard’ diagnostic test, this uniform case definition has now become a new ‘gold standard’ for TBM diagnostic test comparison; despite this not being its purpose.

2.1.5.3 Possible TBM

Within the uniform case definition, what constitutes a ‘diagnosis of TBM’ for the purpose of a reference standard may differ; this was not defined by the authors as this practice was not intended. Given the intention of a reference standard should be to select all cases of a disease (i.e. TBM), and no cases without the disease, common approaches have been to combine ‘definite and probable TBM’, [67] or to combine ‘definite, probable and possible TBM’, [37,65] and label these groups as having a diagnosis of TBM.

The inclusion of individuals with ‘possible TBM’ in a TBM reference standard ensures all ‘true’ cases of TBM are likely to be included in the standard; however, some diseases other than TBM are also likely to be included. This is a particular issue in HIV co-infected TBM, where HIV-associated clinical data and CSF parameters may build up the numerical diagnostic score to a value close to triggering a diagnosis of at least possible TBM. Inclusion of possible TBM in a reference standard may artificially lower sensitivity of the index test (i.e. the test being evaluated); any non-TBM cases will test negative by the index test, and this will reduce its sensitivity. Yet exclusion of possible TBM from a reference standard may artificially elevate test sensitivity, given the possible TBM group likely includes some TBM cases, possibly those with lower bacillary load. A definite plus probable TBM reference standard may select a reference population with higher bacillary load,

therefore creating more chance of index test positivity. The true sensitivity may lie in between the sensitivity values from each of these two reference standards. As such, presentation of index test data against multiple standards is commonplace.

2.1.5.4 Inclusion of the index test in a reference standard

Another consideration with TBM reference standards is whether the index test (e.g. Xpert) should be included in the reference standard. This confusion may arise from the presence in the uniform case definition of CSF NAAT (e.g. Xpert) as a criterion for definite TBM (when positive). The greatest impact of including the index test in a reference standard is that specificity cannot be accurately assessed; any false positive result would not be detected when the index test forms part of the reference standard, as the result would instead be labelled a true positive. This is particularly a risk if the index test is more sensitive than any other aspect of the reference standard, i.e. the index test (within the reference standard) is the only positive component of that reference standard. When assessing molecular tests in CSF, false positive *M. tuberculosis* results are likely exceptional; the CSF is highly unlikely to contain DNA of *M. tuberculosis* that is not relevant to the clinical presentation. However, to ensure accurate sensitivity data, an index test should be excluded from a reference standard against which it is being compared.

2.1.6 GeneXpert MTB/RIF Ultra

2.1.6.1 A new generation of Xpert

In recent years a new generation of Xpert has become commercially available for *M. tuberculosis* detection; Ultra. Ultra aims to improve the sensitivity of TB diagnosis and enhance rifampicin resistance identification, using the same cartridge based nested PCR amplification as Xpert. A larger reaction chamber, plus incorporation of two different multicopy amplification targets (*IS6110* and *IS1081*), intend to improve test sensitivity through a reduced limit of detection of bacterial colony forming units.[70] Adaptation of molecular probes and testing approach are designed to differentiate between silent mutations (those not conferring resistance), and mutations conferring resistance.[71] Whereas Xpert has a limit of detection of 130 CFU/mL, Ultra's limit of detection is approximately 10 CFU/mL.[62] Given this lower limit of *M. tuberculosis* detection, a new semi-quantification category (trace) has been added to Ultra. This trace category, the lowest possible positive detection category, adds to existing categories of 'high' (rarely seen in TBM), 'medium', 'low' and 'very low' *M. tuberculosis* detection.

2.1.6.2 GeneXpert MTB/RIF Ultra for pulmonary tuberculosis

Ultra has already shown its diagnostic superiority in pulmonary TB. In a prospective multicentre study of adults with pulmonary TB (n=1520), comparing Ultra vs. Xpert testing of sputum samples, Ultra demonstrated a 4.9% increase in sensitivity over Xpert (sensitivities; 87.8 vs. 82.9%, respectively), with an associated 3.2% decrease in specificity (specificities; 94.8 vs. 98%, respectively).[70] Additionally, in this study Ultra had a higher diagnostic sensitivity than Xpert; 63 vs. 46% in smear negative, culture positive sputum samples (n=135); and 90 vs. 77% in culture positive sputum samples from HIV co-infected individuals (n=115).[173] However, no improvement in sensitivity was seen amongst a subgroup of HIV-uninfected individuals (test sensitivities 91 vs. 90%, Ultra and Xpert respectively), and Ultra specificity was lower than Xpert.

2.1.6.3 GeneXpert MTB/RIF Ultra for the diagnosis of tuberculous meningitis

In 2018 a study comparing Ultra and Xpert for the diagnosis of TBM was published,[67] subsequently followed by three studies evaluating the role of Ultra in the diagnosis of extrapulmonary TB,[72,174,175] inclusive of varying numbers of CSF samples. In the 2018 TBM study, frozen CSF was stored from patients screened for a trial of the treatment of HIV co-infected cryptococcal meningitis.[67] In 23 HIV co-infected individuals subsequently diagnosed with definite or probable TBM, pre-centrifuged cryopreserved CSF was thawed and then tested with Ultra and Xpert. Ultra and Xpert sensitivities were 69.6% (16/23) and 43.5% (10/23) respectively, compared with a reference standard of definite or probable TBM. Of 21 cases positive by Ultra, Ultra was the only positive mycobacterial test in 8 cases, suggesting Ultra may detect cases of TBM below the threshold of detection of other confirmatory mycobacterial tests. An additional, highly impressive, diagnostic sensitivity of 95% was reported when Ultra (the index test) was included in the reference standard; all 8 cases where Ultra was the only positive test were thus given a final diagnosis of 'definite TBM' on the basis of Ultra alone. This inclusion of Ultra in the reference standard assumes these 8 cases were not false positive diagnoses. Of these 8 cases positive only by Ultra, 4 died, 1 was lost to follow up, and a further 2 died on treatment. Certainty of TBM was lacking in these 8 cases, and the possibility of false positives cannot be rejected entirely. Subsequent to this study the WHO recommended Ultra replace Xpert in all settings.[167]

In the three additional studies comparing Ultra and Xpert testing of extrapulmonary samples, 43, 4 (retrospectively tested), and 16 (prospectively tested) CSF samples of suspected TBM cases were included. The study containing the largest group (n=43) used at least 3mls of uncentrifuged CSF for testing.[72] Sensitivities of Ultra and Xpert were 44.2% (19/43) vs. 18.6% (8/43) respectively,

p=0.01. Whilst the reported sensitivity of Ultra was higher when testing bacteriologically confirmed TBM; 86.4% (19/22) vs. 36.4% (8/22) respectively, p=0.001, cases positive only by NAAT were included in the reference standard. Of the 43 cases, all were smear negative and only 3 were culture positive.

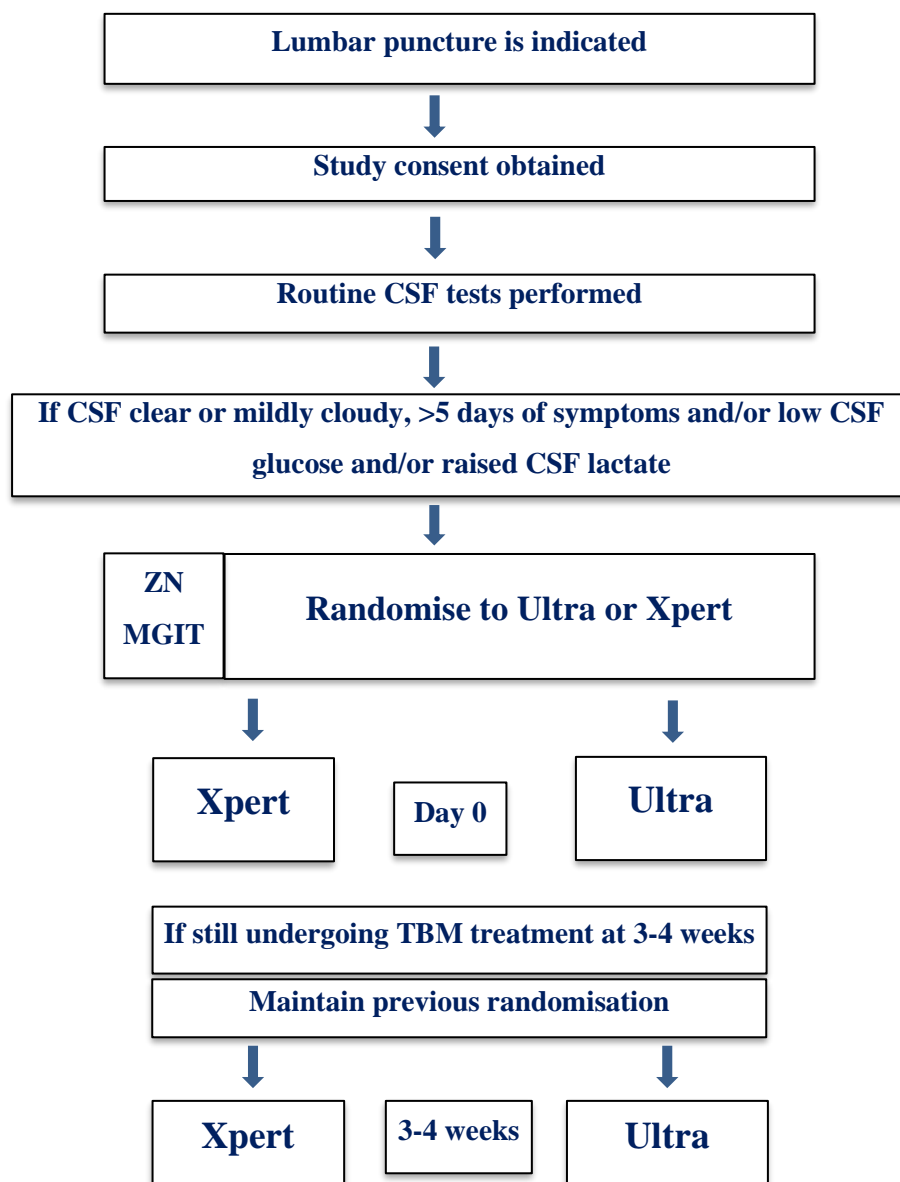
A larger evidence base is required to support Ultra for the diagnosis of TBM, and assess superiority of Ultra over Xpert. No randomised comparison of Ultra and Xpert had been performed at the time of conducting the study that will now be described.

2.2 Methods

2.2.1 Study design

This study was a prospective randomised diagnostic study comparing the diagnostic performances of Ultra and Xpert for the diagnosis of TBM. Enrolled participants were randomised (1:1) to receive baseline CSF testing by either Ultra or Xpert (figure 2-1).

Figure 2-1: Study flow



CSF=cerebrospinal fluid. MGIT=mycobacterial growth indicator tube. TBM=tuberculous meningitis. Ultra=GeneXpert MTB/RIF Ultra. Xpert=GeneXpert MTB/RIF. ZN=Ziehl-Neelsen staining.

In addition, repeat Ultra or Xpert testing was performed on routine follow up CSF sampled at 3-4 weeks, using the allocation test (Ultra or Xpert) following the initial randomisation. The rationale for a randomised study design, rather than using both tests for each CSF sample, was as follows: TBM is associated with very low numbers of *M. tuberculosis* bacteria in CSF, and the performance of tests that detect *M. tuberculosis* are dependent on the CSF volume tested. Using large CSF

volumes (≥ 6 mls), and randomising to one of Ultra or Xpert, provides estimates of the diagnostic performances of these two tests matching clinical practice. Halving the CSF sample (and the *M. tuberculosis* bacteria) to perform both Ultra and Xpert reduces the sensitivity of both tests. Randomisation was performed using a randomisation list generated by a program written in R (version 3.4).

2.2.2 Study participants

Study participants were enrolled into a prospective study of brain infection in Vietnamese adults, with this comparison of Ultra and Xpert forming part of that study. Participants were ≥ 16 years old, with suspected TBM based on clinical and CSF findings (clear or mildly cloudy CSF, in addition to one of >5 days of symptoms, low CSF glucose, or raised CSF lactate), based at HTD, Ho Chi Minh City, Vietnam. Patients were excluded from enrollment if a lumbar puncture was contraindicated or if informed consent was not given by the patient (or by a relative if the patient did not have capacity). Ethical approvals for this study were obtained from the Oxford Tropical Research Ethics Committee (OxTREC) (16-17), and the ethical committee of HTD (27/ HDDD). This study was funded by the Wellcome Trust (grant numbers 204904/Z/16/Z and 106680/B/14/Z) and the Foundation for Innovative New Diagnostics (FIND) who supplied test cartridges and logistics.

2.2.3 Cerebrospinal fluid

2.2.3.1 Processing

At least 6mls of CSF (if available) was sampled by lumbar puncture and used for dedicated mycobacterial tests. No lower limit of CSF volume was set for study entry however, and if <6 mls was available ZN smear, NAAT (Ultra or Xpert), and MGIT were still performed. All CSF volumes were recorded. Approximately 2-3mls of CSF was sent for bacterial and fungal stain, microscopy and culture, and viral PCR. CSF for mycobacterial testing was centrifuged at 3,000g (4000rpm in a large centrifuge or 6500rpm in an Eppendorf centrifuge) for 15 minutes. All supernatant except 500 μ L was removed, and the deposit was resuspended in the remaining 500 μ L. 100 μ L resuspended deposit was used for ZN smear, 200 μ L for mycobacterial culture (MGIT), and 200 μ L for either Ultra or Xpert.

2.2.3.2 Testing

CSF ZN smear, Xpert and MGIT culture were performed as previously described.[37] Briefly, CSF ZN smears were prepared by placing two drops of CSF deposit placed on a cleaned slide, and

performing hot ZN staining according to local procedures.[37] Slides were examined until positive (single AFB identified) or for up to 30 minutes.

For Xpert 2mls of reagent was added to a 200µl sample in a sterile container. After 5 minutes the sample was shaken gently. This sample was inserted into an entry port in the bottom of the Xpert cartridge. The cartridge was inserted into the Xpert machine and the test was started. For MGIT testing, CSF deposit was added to a MGIT tube containing MGIT supplement (antibiotics and growth supplements) and incubated in a BACTEC MGIT960 system (Becton, Dickinson, New Jersey, USA) until automatically identified as positive or for 56 days. Positive MGIT cultures were tested for susceptibility to rifampin, isoniazid, streptomycin, and ethambutol using a BACTEC MGIT SIRE kit (Becton, Dickinson, New Jersey, USA).

Ultra testing procedure was identical to that of Xpert, with the exception of a different test cartridge. Both Ultra and Xpert were conducted with laboratory technicians blinded to the patient's clinical characteristics.

2.2.4 Data collection

2.2.4.1 Clinical data

Clinical data was collected to establish a 'final diagnosis' for each participant using the uniform case definition for TBM.[69] Demographic data (age, sex), GCS, MRC TBM grade, presenting symptoms and signs, and relevant past medical history were recorded at baseline. CSF parameters, including white cell count, white cell differential, protein, glucose (paired with serum glucose), and lactate were recorded. Results of baseline chest X-ray and brain imaging were recorded. HIV testing was not mandatory for this study; testing was performed when clinically indicated. In practice most participants with suspected TBM undergo HIV testing. Participants reported as HIV negative had a negative test for HIV. Participants reported as HIV positive had either a confirmatory test for HIV, or an already established diagnosis of HIV.

2.2.4.2 Assigning TBM diagnosis

Final TBM diagnoses were assigned following the uniform case definition for TBM clinical research (appendix A).[69] Patients with probable and possible diagnoses were reassigned to not TBM if the treating clinician did not consider the final diagnosis to be TBM and the patient recovered without anti-TB chemotherapy. Final reference standard TBM diagnoses were assigned without the Ultra or Xpert result contributing to the final diagnosis (i.e. reference standards did not include the index test).

2.2.5 Evaluating test performance

Test diagnostic performances (sensitivity, specificity, positive predictive value, and negative predictive value) of Ultra, Xpert, ZN smear and MGIT, were compared against a clinical reference standard (the uniform case definition for TBM),[69] and against a mycobacterial culture reference standard. Definite, probable and possible TBM, definite and probable TBM, and definite TBM alone, were all used as clinical reference standards against which to compare diagnostic tests.

In addition, the diagnostic performances of Ultra and Xpert were evaluated by HIV co-infection status, given higher Xpert sensitivity has been reported in HIV co-infected TBM.[37] Test performances after 3-4 weeks anti-TB chemotherapy were also evaluated on routine follow up CSF samples against the uniform case definition for TBM. Diagnostic performances of rifampicin resistance prediction were assessed against a reference standard of phenotypic DST (for MGIT-positive cases). Semi-quantification of CSF bacterial load was compared between Ultra and Xpert positive cases, using high, medium, low, very low and trace categories. Additionally, an assessment of Ultra and Xpert performance was performed with tested CSF volume divided into 3 groups; >5mls, >2mls and ≤5mls, and ≤2mls.

2.2.6 Statistical analysis

2.2.6.1 Sample size calculation

The previously described Ultra study[67] by Bahr et al suggested Ultra was 25% more sensitive than Xpert for the diagnosis of TBM. Assuming a sensitivity of Xpert of 60%, [37] using a sample size calculation for binary outcomes, a significance level of 5%, and 80% power, a calculated 49 patients with TBM were required in each of the testing arms to be able to detect a 25% difference in sensitivity if it existed. In addition to ~100 participants with TBM, 100 non-TBM brain infection ‘controls’ were tested to generate specificity data. The specificity of Ultra for *M. tuberculosis* detection in CSF is unknown, however a study of Ultra testing for *M. tuberculosis* in sputum samples of patients with pulmonary TB demonstrated a 3.2% decrease in specificity with Ultra compared to Xpert (Ultra 94.8%, Xpert 98%).[173]

2.2.6.2 Recruitment

Approximately 100 cases of TBM present to HTD each year. Based upon Xpert testing practice (i.e. those referred for testing) during a previous study at HTD[37] it was expected that in approximately 50% of cases where CSF Xpert testing was requested by a treating clinician, the patient would be

confirmed as having definite, probable or possible TBM. This enables recruitment to both TBM and control testing groups.

2.2.6.3 Statistical tests

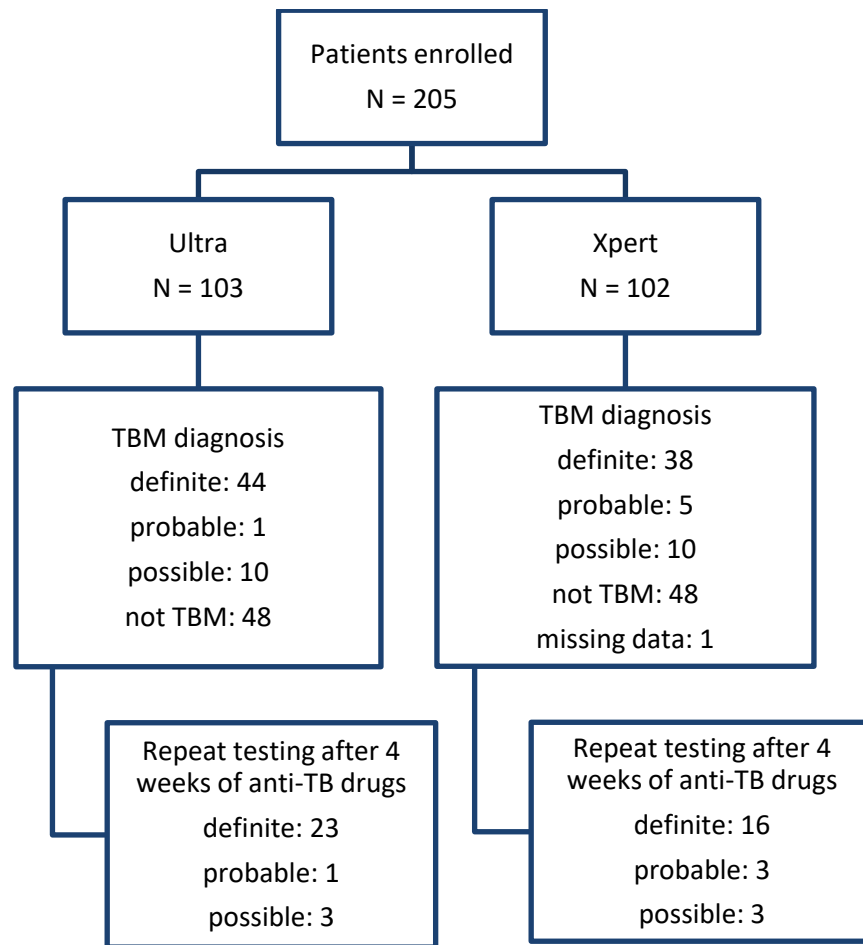
Test performance (sensitivity, specificity, positive and negative predictive values) with associated Wilson CIs were calculated for Ultra and Xpert and compared with those for ZN smear and MGIT. Study tests were compared using the Chi-squared test. Statistical analysis was performed using the programming language R (version 3.5.1).

2.3 Results

2.3.1 Study population

From October 2017 to February 2019, 205 participants were consecutively enrolled into this study and randomised to CSF testing by Ultra (n=103) or Xpert (n=102) (figure 2-2). 204/205 participants were assigned a final diagnosis; 82 (40.2%) definite TBM, 6 (2.9%) probable TBM, 20 (9.8%) possible TBM, and 96 (47.1%) not TBM. Repeat testing was performed after 3-4 weeks of anti-TB chemotherapy in participants with a diagnosis of TBM in whom repeat CSF was available for testing.

Figure 2-2: Study enrolment



Study enrolment by randomisation arm, with final diagnosis shown for both initial and follow up testing. TBM=tuberculous meningitis; Ultra=GeneXpert MTB/RIF Ultra; Xpert=GeneXpert MTB/RIF; ZN=Ziehl-Neelsen

2.3.2 Baseline characteristics

Baseline characteristics of enrolled participants stratified by randomisation arm are shown in table 2-1. Median age was 42 years (Ultra: 42 years; Xpert: 44 years), and 62.9% of participants were male (Ultra: 60.2%; Xpert: 65.7%). Baseline variables in the Ultra and Xpert arms were well matched. Median CSF volumes of 5.8mls CSF (interquartile range [IQR] 5.0-6.0mls) and 5.5mls CSF (IQR 5.0-6.0mls) were used for mycobacterial testing in each of the arms. Median days to MGIT positivity in the Ultra and Xpert arms were 13 (IQR 10-17) and 15 (IQR 13-20), respectively, $p=0.09$. Disease severity (MRC TBM grade 3 in 25.5% [14/55] vs. 28.3% [15/53]), HIV status (HIV positivity in 16.5% [17/103] vs. 14.1% [14/99]), and CSF parameters (CSF WCC

310 per mm³[172-597] vs. 334 per mm³[120-484], CSF:blood glucose ratio 0.35 [0.22-0.46] vs. 0.35 [0.25-0.43]) also appeared well matched between the two arms.

Table 2-1: Baseline characteristics of enrolled participants

	Total no	All patients	Total no	Ultra	Total no	Xpert
Age (years) (Median[IQR])	205	42 (31-57)	103	42 (31-57)	102	44 (33-58)
Sex (male) (No [%])	205	129 (62.9%)	103	62 (60.2%)	102	67 (65.7%)
Final diagnosis (No [%])	204		103		101	
Definite TBM		82 (40.2%)		44 (42.7%)		38 (37.6%)
Probable TBM		6 (2.9%)		1 (1.0%)		5 (5.0%)
Possible TBM		20 (9.8%)		10 (9.7%)		10 (9.9%)
Not TBM		96 (47.1%)		48 (46.6%)		48 (47.5%)
TBM MRC Grade * (No [%])	108		55		53	
1		32 (29.6%)		19 (34.5%)		13 (24.5%)
2		47 (43.5%)		22 (40.0%)		25 (47.2%)
3		29 (26.9%)		14 (25.5%)		15 (28.3%)
HIV status (No [%])	202		103		99	
Positive						
Negative		31 (15.3%)		17 (16.5%)		14 (14.1%)
Unknown		96 (47.5%)		48 (46.6%)		48 (48.5%)
		77 (38.1%)		38 (36.9%)		37 (37.4%)
CSF characteristics * (Median[IQR])						
CSF WCC (per mm ³)	108	332 (142-529)	55	310 (172-597)	53	334 (120-484)
CSF lymphocyte (%)	107	74 (41-87)	55	76 (38-88)	52	74 (41-86)
CSF protein (g/L)	108	1.90 (1.14-2.85)	55	1.87 (1.12-2.79)	53	1.96 (1.31-2.92)

CSF:blood glucose ratio	108	0.35 (0.25-0.3)	55	0.35 (0.22-0.46)	53	0.35 (0.25-0.43)
CSF volume for mycobacterial tests (mls) (Median[IQR])	201	5.8 (5.0-6.0)	100	5.8 (5.0-6.0)	101	5.5 (5.0-6.0)

* MRC TBM grade and CSF characteristics shown only for 108 patients with definite, probable or possible TBM. CSF=cerebrospinal fluid; HIV=human immunodeficiency virus; IQR=interquartile range; MRC=Medical Research Council; MGIT=mycobacteria growth indicator tube; TBM=tuberculous meningitis; Ultra=GeneXpert MTB/RIF Ultra; WCC=white cell count; Xpert=GeneXpert MTB/RIF

2.3.3 Diagnostic performance

The diagnostic performances of Ultra, Xpert, ZN smear and MGIT against a reference standard of definite, probable and possible TBM, and against a reference standard of definite and probable TBM, are shown in table 2-2. The diagnostic sensitivities of Ultra and Xpert against a reference standard of definite, probable and possible TBM were 47.2% (25/53, 95% CI 34.4-60.3%) and 39.6% (21/53, 95% CI 27.6-53.1%) respectively (p=0.56). Specificities of Ultra and Xpert were each 100% (44/44, 95% CI 92.0-100% and 48/48, 95% CI 92.6-100%, respectively). Against the same definite, probable and possible TBM reference standard sensitivities of ZN smear and MGIT were 71.3% (77/108, 95% CI 62.5-79.0%) and 47.9% (45/94, 95% CI 38.1-57.9%) respectively. Against a MGIT reference standard, sensitivities of Ultra and Xpert were 90.9% (20/22, 95% CI 72.2-97.5%) and 81.8% (18/22, 95% CI 61.6-92.7) and specificities were 93.9% (62/66, 95% CI 85.4-97.6%) and 96.9% (63/65, 95% CI 89.5-99.2%) respectively. The diagnostic sensitivities of Ultra and Xpert against a reference standard of definite plus probable TBM were 58.1% (25/43, 95% CI 43.3-71.6%) and 48.8% (21/43, 95% CI 34.6-63.2%) respectively. The diagnostic sensitivities of Ultra and Xpert against a reference standard of definite TBM alone were 59.5% (25/42, 95% CI 44.5-73.0%) and 55.3% (21/38, 95% CI 39.7-69.9%), p=0.87.

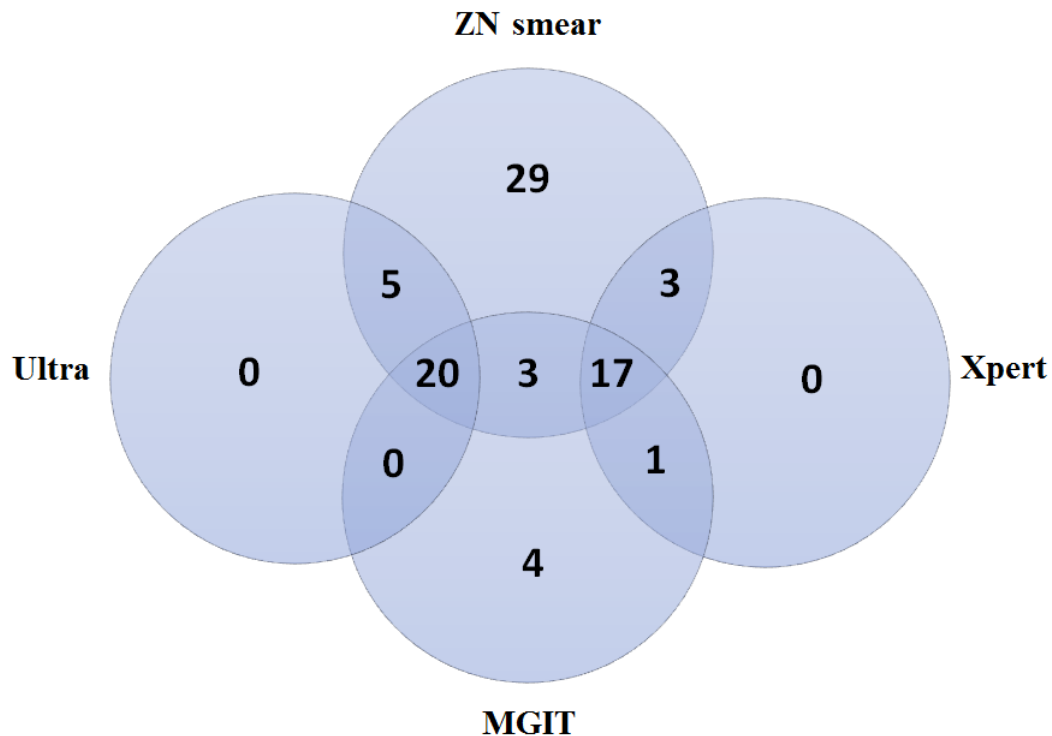
Table 2-2: Diagnostic performances against a clinical reference standard

	Ultra	Xpert	ZN smear	MGIT culture
Reference standard: definite, probable and possible TBM				
Positive tests	25/53*	21/53	77/108	45/94
Sensitivity (95% CI)	47.2% (34.4-60.3%)	39.6% (27.6-53.1%)	71.3% (62.5-79.0%)	47.9% (38.0-57.9%)
Specificity (95% CI)	100% (92.0-100%)	100% (92.6-100%)	100% (96.1-100%)	100% (95.6-100%)
PPV (95% CI)	100% (86.7-100%)	100% (84.5-100%)	100% (95.2-100%)	100% (92.1-100%)
NPV (95% CI)	61.1% (49.6-71.5%)	60.0% (49.0-70.0%)	72.2% (67.2-82.1%)	63.2% (54.7-70.9%)
Reference standard: definite and probable TBM				
Positive tests	25/43	21/43	77/88	45/75
Sensitivity (95% CI)	58.1% (43.3-71.6%)	48.8% (34.6-63.2%)	87.5% (79.0-92.9%)	60.0% (48.7-70.3%)
Specificity (95% CI)	100% (93.4-100%)	100% (93.8-100%)	100% (96.8-100%)	100% (96.4-100%)
PPV (95% CI)	100% (86.7-100%)	100% (84.5-100%)	100% (95.2-100%)	100% (92.1-100%)
NPV (95% CI)	75.0% (63.9-83.6%)	72.5% (61.9-81.1%)	91.3% (85.0-95.1%)	77.4% (69.6-83.7%)

* Of 55 cases of definite probable or possible TBM tested by Ultra, 2 returned an error result. Therefore only 53 cases are included in the sensitivity calculation. CI=confidence interval; MGIT=mycobacteria growth indicator tube; NAAT=nucleic acid amplification test; NPV=negative predictive value; PPV=positive predictive value; Ultra=GeneXpert MTB/RIF Ultra; Xpert=GeneXpert MTB/RIF; ZN=Ziehl-Neelsen

Figure 2-3 shows positive mycobacterial tests in individuals with at least one confirmatory test for TBM. In this Venn diagram the overlap of positive CSF Ultra, Xpert, smear and culture are shown, and cases identified where only one confirmatory mycobacterial test was positive. In addition to positives and negatives, six error results were returned in the Ultra arm, and none in the Xpert group. Eight MGIT samples showed contaminated growth.

Figure 2-3: Positive mycobacterial tests in individuals with at least one confirmatory test for tuberculous meningitis



168 positive mycobacterial tests (25 positive Ultra, 21 positive Xpert, 77 positive ZN smear, 45 positive MGIT) from 82 patients with a diagnosis of definite TBM. MGIT=mycobacteria growth indicator tube; Ultra: GeneXpert Ultra MTB/RIF; Xpert: GeneXpert MTB/RIF; ZN: Ziehl-Neelsen

2.3.4 Diagnostic performance in HIV co-infection

HIV testing was performed in 127/205 (62.0%) participants, and in 100/108 (92.6%) participants with TBM. HIV co-infection was diagnosed in 31 cases (17/65 Ultra and 14/62 Xpert tested participants). A comparison of diagnostic performances in HIV co-infected and HIV-uninfected participants against a reference standard of definite, probable and possible TBM is shown in table 2-3. The diagnostic sensitivities of Ultra and Xpert in HIV-uninfected participants against this

reference standard were 38.9% (14/36, 95% CI 24.8-55.1%) and 22.9% (8/35, 95% CI 12.1-35.0%) respectively (p=0.23). The specificities of Ultra and Xpert in HIV-uninfected participants were 100% (0/9, 95% CI 70.1-100% and 0/13, 95% CI 77.2-100%, respectively).

The diagnostic performances of Ultra and Xpert were additionally compared against a reference standard of definite plus probable TBM in HIV co-infected participants, for direct comparison against test performance data published by Bahr et al.[67] Diagnostic sensitivities of Ultra and Xpert against this reference standard were 81.8% (9/11, 95% CI 52.3-94.9%) and 83.3% (10/13, 95% CI 55.2-95.3%) respectively (p=1.0). Specificities of Ultra and Xpert were both 100% (0/6, 95% CI 61.0-100% and 0/1, 95% CI 34.2-100%, respectively). The diagnostic sensitivities of Ultra and Xpert were higher in HIV co-infected cases than in HIV uninfected cases; 64.3%, 9/14, 95% CI 38.8-83.7% vs. 38.9%, 14/36, 95% CI 24.8-55.1% for Ultra, and 76.9%, 10/13, 95% CI 49.7-91.8% vs. 22.9%, 8/35, 95% CI 12.1-39.0% for Xpert, respectively, against a reference standard of definite probable and possible TBM.

Table 2-3: Diagnostic performance of Ultra and Xpert against a clinical reference standard, by HIV co-infection status

	Ultra		Xpert	
	HIV negative	HIV positive	HIV negative	HIV positive
Reference standard: definite, probable and possible TBM				
Positive tests	14/36	9/14	8/35	10/13
Sensitivity (95% CI)	38.9% (24.8-55.1%)	64.3% (38.8-83.7%)	22.9% (12.1-39.0%)	76.9% (49.7-91.8%)
Specificity (95% CI)	100% (70.1-100%)	100% (43.9-100%)	100% (77.2-100%)	100% (20.7-100%)
PPV (95% CI)	100% (78.5-100%)	100% (70.1-100%)	100% (67.6-100%)	100% (72.2-100%)
NPV (95% CI)	29.0% (16.1-46.6%)	37.5% (13.7-69.4%)	32.5% (20.1-48.0%)	25.0% (4.6-69.9%)
Reference standard: definite and probable TBM				
Positive tests	14/29	9/11	8/27	10/12
Sensitivity (95% CI)	48.3% (31.4-65.6%)	81.8% (52.3-94.9%)	29.6% (15.9-48.5%)	83.3% (55.2-95.3%)
Specificity (95% CI)	100% (80.6-100%)	100% (61.0-100%)	100% (84.5-100%)	100% (34.2-100%)
PPV (95% CI)	100% (78.5-100%)	100% (70.1-100%)	100% (67.6-100%)	100% (72.2-100%)
NPV (95% CI)	51.6% (24.8-68.0%)	75.0% (40.9-92.9%)	52.5% (37.5-67.1%)	50.0% (15.0-85.0%)

CI: confidence interval; NAAT: nucleic acid amplification test; NPV: negative predictive value; PPV: positive predictive value; TBM: tuberculous meningitis; Ultra: GeneXpert MTB/RIF Ultra; Xpert: GeneXpert MTB/RIF

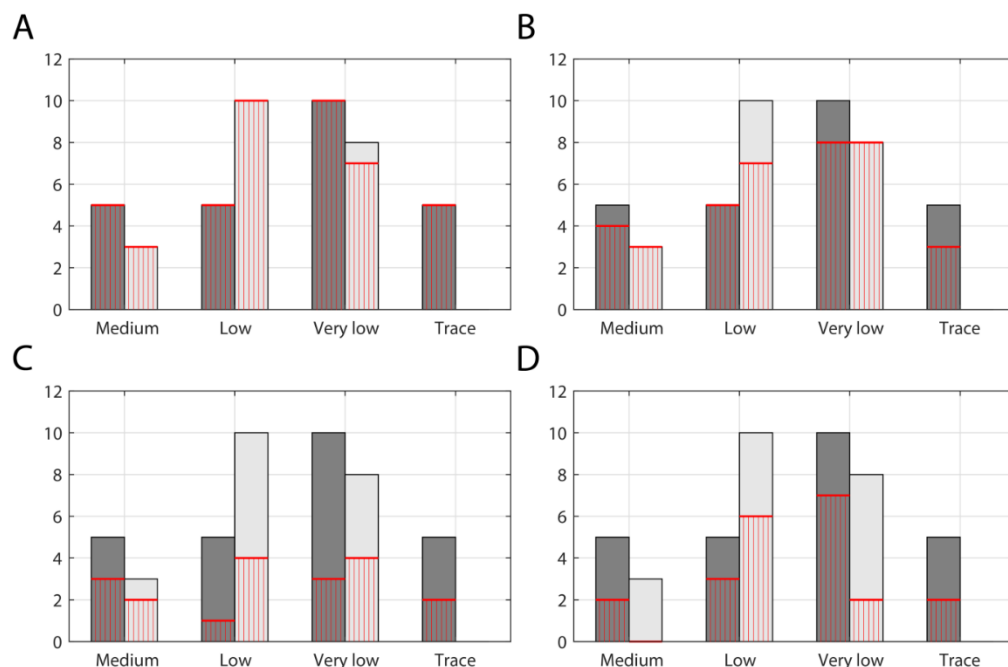
2.3.5 Identification of rifampicin resistance

Rifampicin resistance was detected in 17.4% (8/46) cases where NAAT was positive; 5/25 (20.0%) Ultra, 3/21 (14.3%) Xpert. An additional 5 cases ‘trace’ positive by Ultra returned a result of ‘indeterminate resistance’, the resistance result given for this semi-quantification category. Rifampicin resistance testing was negative in 71.7% (33/46) cases where NAAT was positive. Of 45 positive MGIT cultures, 8 showed rifampicin resistance by phenotypic DST, all of which were detected by Xpert or Ultra (5 Ultra, 3 Xpert).

2.3.6 Semi-quantification of CSF bacterial numbers by Ultra and Xpert

Xpert can categorise specimen bacterial numbers into high, medium, low or very low. Ultra has an additional ‘trace’ category. High bacterial numbers are rare in TBM and were not seen in this study. Semi-quantification categories obtained from the CSF are shown in figure 2-4. Of those cases positive by Ultra, 15/25 (60.0%) cases were categorised as containing very low or trace numbers of bacteria, compared with 8/21 (38.1%) samples with very low bacterial numbers detected by Xpert.

Figure 2-4: Semi-quantification of positive Ultra and Xpert results

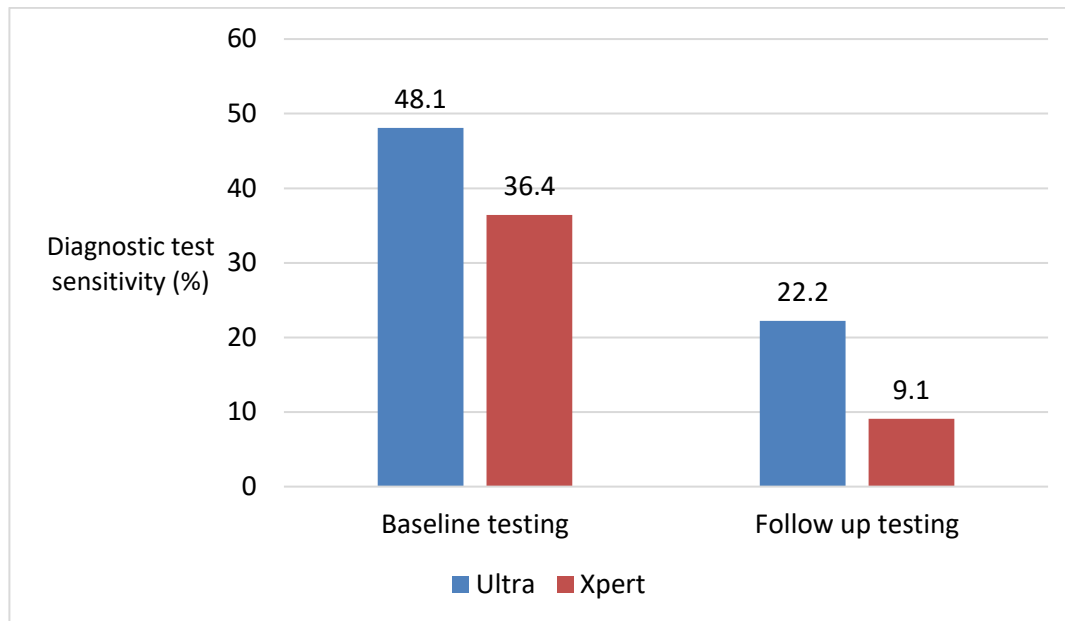


Semi-quantification results for positive Ultra (dark shading, n=25) and Xpert (light shading, n=21). The filled shaded areas represent all positive tests in that semi-quantification category. The patterned (vertical lined) area in each bar represents the number of those cases also positive by ZN smear (A), MGIT (B), or that have (C) or do not have (D) HIV co-infection. Semi-quantification results for Ultra were: medium 5/25 (20.0%); low 5/25 (20.0%); very low 10/25 (40.0%); trace 5/25 (20.0%). Semi-quantification results for Xpert were: medium 3/21 (14.3%); low 10/21 (47.6%); very low 8/21 (38.1%). Ultra=GeneXpert MTB/RIF Ultra; Xpert=GeneXpert MTB/RIF. Dr Vinh Dao Nguyen assisted with the design of this figure.

2.3.7 Diagnostic performance after the start of anti-TB chemotherapy

Routine follow up CSF was sampled and tested in 49 participants treated for TBM (27 Ultra, 22 Xpert). A median of 5.5mls CSF was tested in each of the Ultra and Xpert arms. In these 49 participants, 48.1% (13/27) of the Ultra arm had a positive test and 36.4% (8/22) of the Xpert arm at presentation. After a mean of 27 (Ultra) and 28 (Xpert) days of anti-TB chemotherapy, 22.2% (6/27) of the Ultra arm had a positive test versus 9.1% (2/22) of the Xpert arm (figure 2-5).

Figure 2-5: A comparison of the diagnostic sensitivities of Ultra and Xpert between baseline and testing after 3-4 weeks anti-tuberculosis chemotherapy



Data shown for patients undergoing both baseline and follow up CSF sampling (n=49; Ultra 27, Xpert 22). Ultra and Xpert sensitivities shown against a reference standard of definite, probable or possible TBM. All cases with a positive NAAT at follow up testing (n=8), were positive at baseline testing. NAAT=nucleic acid amplification test; Ultra=GeneXpert MTB/RIF Ultra; Xpert=GeneXpert MTB/RIF

2.3.8 CSF parameters in individuals undergoing repeat diagnostic testing

CSF parameters are shown for 44/49 patients who underwent repeat diagnostic testing (25/27 Ultra, 19/22 Xpert), for whom non-mycobacterial CSF parameters were available at both time points (table 2-4). At repeat testing, CSF parameters were generally worse (elevated WCC, protein and lactate) in those individuals who re-tested as positive in both Ultra and Xpert groups, compared with those who re-tested as negative.

Compared with baseline testing, CSF parameters of individuals positive by Ultra were generally improved (reduced WCC and lactate, and increased CSF: blood glucose ratio). Compared with baseline testing, CSF parameters of individuals positive by Xpert were generally worse (elevated WCC, protein and lactate). Whilst the number of cases positive at repeat testing is small, these results may support a proposed role for Ultra in the detection of cases with lower bacillary load (where repeat CSF parameters for cases positive at repeat testing by Ultra are generally improved compared with repeat CSF parameters for cases positive at repeat testing by Xpert).

Table 2-4: CSF parameters in individuals undergoing repeat diagnostic testing

	Ultra			Xpert		
Median values	Baseline	Repeat		Baseline	Repeat	
		Positive tests	Negative tests		Positive tests	Negative tests
	N=25	N=6	N=19	N=19	N=2	N=17
CSF WCC per mm ³) (95% CI)	340 (207-623)	136 (63-241)	70 (42-126)	144 (104-422)	314 (240-389)	73 (35-262)
Lymphocyte (%) (95% CI)	82 (28-88)	60 (45-73)	87 (84-89)	80 (67-87)	64 (64-65)	87 (67-89)
Protein (g/L) (95% CI)	1.66 (1.11-2.75)	1.93 (1.30-2.38)	0.81 (0.44-1.52)	2.12 (1.64-2.88)	6.03 (5.12-6.93)	1.61 (0.60-2.41)
CSF/blood glucose ratio (95% CI)	0.31 (0.20-0.40)	0.37 (0.32-0.78)	0.52 (0.45-0.62)	0.34 (0.25-0.39)	0.47 (0.40-0.51)	0.43 (0.40-0.51)
Lactate (mmol/L) (95% CI)	4.31 (3.40-7.60)	4.12 (3.35-4.67)	2.87 (2.54-3.40)	3.89 (3.27-5.50)	5.13 (4.62-5.63)	3.54 (3.20-5.19)

CI=confidence interval; CSF=cerebrospinal fluid; Ultra=GeneXpert MTB/RIF Ultra; WCC=white cell count; Xpert=GeneXpert MTB/RIF

Analysing only those participants testing positive by Ultra or Xpert at presentation, 38.5% (5/13) in the Ultra arm were still positive after 4 weeks treatment, compared with 25.0% (2/8) in the Xpert arm. The influence of drug resistance on a 4-week positive Ultra or Xpert is shown in table 2-5. Of

those participants with a positive baseline CSF NAAT and positive baseline CSF MGIT (with DST), and a positive follow-up NAAT, 4/5 (80.0%) had an *M. tuberculosis* isolate resistant to at least one first line anti-TB drug. Of those with a positive baseline CSF NAAT and positive baseline CSF MGIT (with DST), and a negative follow-up NAAT, 5/11 (45.6%) had an *M. tuberculosis* isolate resistant to at least one first line anti-TB drug. Anti-TB chemotherapy data were not analysed for this study, therefore adjustment of anti-TB chemotherapy regimens upon discovering drug resistance could not be confirmed, confounding analysis of the effect of drug resistance upon repeat NAAT testing.

Table 2-5: Association between TBM drug susceptibility testing and follow up NAAT testing

NAAT	Baseline CSF NAAT result	Baseline CSF MGIT result	MGIT drug susceptibility testing	Follow up CSF NAAT result	Follow up CSF MGIT result
Ultra	Positive	Positive	RHZES	Positive	Positive
Ultra	Positive	Positive	HZS	Positive	Negative
Ultra	Positive	Positive	S	Positive	Not performed
Ultra	Positive	Negative	Not performed	Positive	Negative
Ultra	Positive	Negative	Not performed	Positive	Negative
Xpert	Positive	Positive	HS	Positive	Negative
Xpert	Positive	Positive	Fully sensitive	Positive	Negative
Ultra	Positive	Positive	RHS	Negative	Negative
Ultra	Positive	Positive	RHS	Negative	Negative
Ultra	Positive	Positive	S	Negative	Negative
Ultra	Positive	Positive	Fully sensitive	Negative	Not performed
Ultra	Positive	Positive	Fully sensitive	Negative	Negative
Ultra	Positive	Positive	Fully sensitive	Negative	Negative
Ultra	Positive	Positive	Fully sensitive	Negative	Negative
Ultra	Positive	Positive	Fully sensitive	Negative	Negative
Xpert	Positive	Positive	HZS	Negative	Negative
Xpert	Positive	Positive	Z	Negative	Contaminated
Xpert	Positive	Positive	Fully sensitive	Negative	Negative
Xpert	Positive	Negative	Not performed	Negative	Negative
Xpert	Positive	Negative	Not performed	Negative	Negative
Xpert	Positive	Positive	Not performed	Negative	Negative

Follow up NAAT testing is shown for samples where baseline NAAT was positive (n=21). Drug susceptibility testing of *M. tuberculosis* isolated from CSF is compared with follow up testing results. With MGIT drug susceptibility testing the listed anti-TB chemotherapy agents are those for which there is resistance in each case. CSF=cerebrospinal fluid; E=ethambutol; H=isoniazid; MGIT=mycobacterial growth indicator tube; NAAT=nucleic acid amplification test; R=rifampicin; S=streptomycin; TBM=tuberculous meningitis; Ultra=GeneXpert MTB/RIF Ultra; Xpert=GeneXpert MTB/RIF; Z=pyrazinamide

2.3.9 Diagnostic performance by CSF volume tested

CSF volume used for mycobacterial testing was divided into 3 categories; >5mls, 2-5mls and ≤ 2 mls, consistent with CSF volume intervals in a previous study.[37] Diagnostic sensitivities of Ultra and Xpert against a reference standard of definite probable and possible TBM were 46.5% (20/43, 95% CI 32.5-61.1%) vs. 36.4% (16/44, 95% CI 23.8-51.1%) for CSF volumes > 5mls, and 40.0% (6/15, 95% CI 19.8-64.3%) vs. 38.5% (5/13, 95% CI 17.7-64.5%) for CSF volumes of 2-5mls. Only three patients with TBM (two definite, one possible) underwent testing of CSF volumes ≤ 2 mls (Ultra; 1/2 positive, Xpert 1/1 positive).

2.4 Discussion

TBM diagnosis continues to challenge clinicians. Currently available diagnostic tests are insufficiently sensitive for *M. tuberculosis* detection in the paucibacillary CSF of TBM. Xpert offers rapid results and detection of rifampicin resistance; both vitally important to guiding early and appropriate anti-TB chemotherapy. However, Xpert has limitations in the diagnosis of TBM.[176] An improved cartridge offering increased diagnostic sensitivity would be hugely welcome.

In this study Ultra was not superior to Xpert for the detection of *M. tuberculosis* in CSF of individuals with TBM, using either clinical or culture reference standards. There was, however, a suggestion that Ultra was able to detect more patients with TBM with very low, or trace, numbers of bacteria in their CSF and that Ultra may be more sensitive than Xpert once anti-TB treatment has started. Modifications to the Ultra cartridge include a larger reaction chamber (allowing more sample for PCR), and the addition of two multicopy genes; both of which aim to improve test sensitivity. These modifications may be insufficient to improve the diagnostic sensitivity of *M. tuberculosis* detection in CSF, and *M. tuberculosis* DNA load may remain below the lower limit of detection of the test in many cases. CSF processing at our site involves large volume CSF sampling (to increase the *M. tuberculosis* load), centrifugation (to concentrate the *M. tuberculosis* in the pellet), followed by the resuspension of 200 μ L of pellet prior to testing. A larger reaction chamber may not further enhance this process.

Ultra showed improved diagnostic sensitivity over Xpert in HIV-uninfected individuals, against all variations of the clinical TBM reference standard (definite probable and possible, definite and probable, or definite alone), and against mycobacterial culture. However, these did not reach statistical significance. In HIV uninfected TBM CSF bacillary load is lower than in HIV co-infected disease.[36] Ultra has already shown promise in the diagnosis of smear negative culture positive,

and HIV co-infected, sputum samples of individuals with pulmonary TB;[173] both situations when the bacillary load is lower.

The diagnostic sensitivity of Ultra was higher for HIV co-infected samples than for HIV uninfected samples, against the composite reference standard or against culture; a pattern also seen with Xpert, ZN smear and culture, and described previously with Xpert.[37] This is likely attributed to a higher bacillary load in HIV co-infected individuals.[36]

In a previous study of 23 HIV co-infected individuals with definite or probable TBM, Ultra showed diagnostic superiority over Xpert (69.6 vs. 43.5% respectively) against a reference standard of definite plus probable TBM.[67] When analysing an HIV co-infected sub-group, against an identical reference standard of definite plus probable TBM, this study reported sensitivities of 81.8 and 83.3% for Ultra and Xpert respectively. These results may be explained by the methodological differences between this study and that by Bahr et al.[67] This study prospectively tested fresh CSF with a freezing and re-thawing process. The effect of freezing CSF on diagnostic sensitivity is unknown. In addition to this, the randomised design of this study ensured CSF pellet was not split between too many tests, instead reflecting testing procedure as it is carried out in current practice. Performing two NAATs on the same CSF sample reduces the bacillary load per sample and reduces sensitivity of all testing methods.

The specificity of Ultra in this study was 100% (as was that of Xpert) when a uniform case definition for TBM was used as a reference standard. NAAT specificity only fell in this study when MGIT was used as a reference standard. In a previous study where Ultra was used to test sputum in suspected pulmonary TB, Ultra showed reduced specificity vs. Xpert; however mycobacterial culture was the reference standard used in that study.[173] A fall in NAAT specificity may be expected when a reference standard of mycobacterial culture is used; culture will only detect viable bacteria, whilst NAAT may detect DNA of dead bacteria which cannot be cultured, leading to false positive NAAT results against a mycobacterial culture reference standard.[177] This study adds important specificity data for Ultra testing of CSF for *M. tuberculosis*. In previously published data describing *M. tuberculosis* detection in CSF with Ultra, of 21 samples positive by Ultra, 8 cases (3 probable TBM, 3 possible TBM, 2 not TBM) were positive by Ultra alone with no additional confirmatory mycobacterial tests testing positive.[67] In this study there were 25 positive Ultra tests, from 55 patients with definite probable or possible TBM who were tested by Ultra. Of these, all 25 (100%) had a positive ZN smear, and 20 (80.0%) had a positive MGIT. No positive Ultra results were recorded in TBM cases with a probable or possible diagnosis of TBM, nor in any cases where another non-TBM diagnosis was confirmed. In the setting of this study Ultra did not

diagnose additional cases of TBM missed by other confirmatory mycobacterial testing methods. This is likely due to the high sensitivity of ZN smear microscopy at our site, where sensitivity of ZN smear is superior to that of Xpert and MGIT.

The strengths of this study are that it is large, prospective and randomised, and includes information on the performance of both tests after the start of anti-TB treatment. Laboratory technicians were blinded to the suspected diagnosis on baseline testing, and testing was performed immediately after randomisation. CSF was sampled, processed and tested the same way for both Ultra and Xpert. Median volumes of 5.8mls and of 5.5mls were used for mycobacterial testing in the Ultra and Xpert arms, respectively. A limitation of the study is that specificity data comes mostly from HIV-uninfected individuals. HIV testing was not mandatory for all patients. Individuals with TBM were routinely tested for HIV following standard ward procedures; however additional non-TBM cases were tested for HIV at the discretion of the treating clinician. Of 96 patients in the 'not TBM' category, from whom specificity data are generated, only 4/96 (4.2%) had HIV co-infection. Therefore, additional data are required to confirm a high specificity of Ultra in HIV co-infection. An additional limitation is that whilst this study was powered to detect a 25% improvement in diagnostic sensitivity with Ultra, it cannot be concluded that Ultra is not superior to Xpert at a lower margin of superiority, given this study was not powered to detect a smaller superiority margin. Likewise, this study was not powered to detect superiority specifically in an HIV-uninfected subgroup.

This study showed that Ultra was not superior to Xpert for the diagnosis of TBM. However, given this study was powered to detect a 25% improvement in diagnostic sensitivity with Ultra it remains possible that Ultra was more sensitive than Xpert at a lower margin of superiority. Xpert and Ultra remain vital parts of the diagnostic arsenal of mycobacterial tests. They offer rapid results with rifampicin resistance predication, yet unfortunately cannot exclude TBM when negative. A rapid high sensitivity diagnostic test for TBM unfortunately remains elusive.

2.5 Publications related to this chapter

Data contained within this chapter were published in the following journal article:

1. A randomised comparison of GeneXpert Ultra MTB/RIF and GeneXpert MTB/RIF for the diagnosis of tuberculous meningitis

Joseph Donovan, Do Dang Anh Thu, Nguyen Hoan Phu, Vu Thi Mong Dung, Tran Phu Quang, Ho Dang Trung Nghia, Pham Kieu Nguyet Oanh, Tran Bao Nhu, Nguyen Van Vinh Chau, Vu Thi Ngoc Ha, Vu Thi Ty Hang, Dong Huu Khanh Trinh, Ronald B. Gekus, Le Van Tan, Nguyen Thuy

Thuong Thuong, Guy E. Thwaites

The Lancet Infectious Diseases. Jan 2020; 20: 308–17

Chapter 3

The influence of *Strongyloides stercoralis* co-infection on the presentation, pathogenesis and outcome of tuberculous meningitis

3.1 Introduction

3.1.1 Background and epidemiology of strongyloidiasis

The soil transmitted helminth *S. stercoralis* is the cause of Strongyloidiasis, a neglected chronic parasitic disease of humans. Found throughout tropical and subtropical regions of the world, including Vietnam, *S. stercoralis* infects an estimated 30-100 million individuals worldwide.[178] *S. stercoralis* larvae are found in faecally polluted moist soil,[179] and barefoot activities such as rice cultivation, cleaning of irrigation ditches, and contact with stagnant water represent risks for acquiring infection.[180] The vast majority of *Strongyloides* species do not affect humans.[181] Human strongyloidiasis is almost entirely caused by infection with *S. stercoralis*, with additional *S. fuelleborni* cases in Africa and Papua New Guinea.[181]

Strongyloidiasis was first described in French troops stationed in Vietnam during the late 19th century.[182] Subsequent studies described *S. stercoralis* infection in prisoners of war in Southeast Asia during the Second World War,[183] and in war personnel who had returned to home countries after previously being stationed in Vietnam.[184]

3.1.2 Epidemiology of strongyloidiasis in Vietnam

Limited data describe the current prevalence of *S. stercoralis* infection in Vietnam. In 2016 Schar et al[179] identified 79 published studies of *S. stercoralis* infection in Southeast Asia, inclusive of only one study originating from Vietnam. This Vietnamese study of children in a southern mountainous ethnic minority commune identified no cases of Strongyloidiasis by stool microscopy testing.[185] Subsequently, a large retrospective serological study in southern Vietnam performed 42,920 *Strongyloides* enzyme linked immunosorbent assay (ELISA) tests.[186] *S. stercoralis* antibodies were detected in 7.4% samples, with male sex (prevalence 8.6% in males vs. 6.6% in females, $p < 0.0001$) and increased age (prevalence 11.2% in >60 years of age vs. 7.1% in <60 years of age, $p < 0.0001$) both risk factors for *S. stercoralis* seropositivity. Selection bias was a major limitation of this study, given its inclusion only of samples from unwell patients or those who chose to submit a sample for testing.

A subsequent serological study assessed *S. stercoralis* positivity in 1340 adults (age range 40-70 years) and 270 children (age range 13-15 years), in four regions of Vietnam.[187] In the adult population seropositivity in Ho Chi Minh City was 18.2% (61/335), with higher seropositivity reported in other country regions.[187] Seropositivity was significantly higher amongst males (33.3%, 223/670) than females (24.9%, 167/670), $p=0.001$. [187] The authors used a commercially available ELISA kit (*S. ratti*, Bordier Affinity Products SA, Crissier, Switzerland) to determine IgG antibodies against *S. stercoralis* (test performance for *S. stercoralis*: 88% sensitivity overall, 77% specificity with sera of patients with other helminthic diseases).[188] Both of these studies reported results of serological testing; it is unlikely seropositivity represents active infection in all cases.

3.1.3 Pathogenesis and life cycle of *S. stercoralis*

S. stercoralis has a unique and complex life cycle, which can be ‘free living’ (outside of the human body after passage of rhabditiform larvae in the stool), and ‘parasitic’ (after penetration of human skin by infective filariform larvae).[189] Outside the body male and female worms reproduce sexually and produce larvae.[190] When filariform larvae penetrate human skin they migrate to the lungs, reaching the small intestine after being coughed up and swallowed, or directly via connective tissue penetration.[189] In the small intestine larvae develop into adult female worms which reproduce asexually.[190] Female worms, threaded in the small intestine epithelium, produce eggs which hatch into non-infective rhabditiform larvae and exit the body in human stool, or transform into infective filariform larvae which may achieve ‘autoinfection’ by penetrating intestinal epithelium or perianal skin of the host.[189,190]

3.1.4 Clinical disease of Strongyloidiasis

Infection with *S. stercoralis* is often asymptomatic. Mild clinical disease may manifest as abdominal symptoms (pain and diarrhoea), cough (as the worm transits the lungs), or as a rash which can be either migratory and urticarial (termed ‘larva currens’), or stationary in the form of urticarial crops.[181]

It is the effect of host immunosuppression on *S. stercoralis* that can lead to acute, severe and sometimes fatal manifestations of Strongyloidiasis. Altered host immune status may produce an increased parasite burden, larval dissemination, the ‘hyperinfection’ syndrome, and/or death.[191] In hyperinfection, blood eosinophil counts are often suppressed.[192] Hyperinfection-precipitating host immunosuppression may be secondary to drugs (particularly corticosteroids), disease states (e.g. haematological malignancy or organ transplantation), or immunosuppressive infections (e.g. human T-lymphotropic virus-1 [HTLV-1]).[191] Although HIV-1 co-infection may produce

advanced immunosuppression, the frequency of life threatening disseminated strongyloidiasis in this patient group is unexpectedly low, suggesting the immune processes suppressed in acquired immunodeficiency syndrome (AIDS) are less important for defense against *S. stercoralis*. [181] Widespread corticosteroid use makes these drugs the most likely inducer of hyperinfection to be encountered by clinicians. Why corticosteroids represent such a strong risk factor for *S. stercoralis* hyperinfection is uncertain; although the drug may directly induce hyperinfection by acting on a receptor found on the L3 larvae of *S. stercoralis*. [193]

3.1.5 Diagnosis of Strongyloidiasis

Diagnosing strongyloidiasis requires either identification of *S. stercoralis* larvae or DNA in clinical samples (stool, upper gastrointestinal tract, pulmonary), or measurement of an immunological response. Diagnosis may be challenging, and current tests have limitations.

3.1.5.1 *S. stercoralis* serology

S. stercoralis ELISA usually detects serum IgG against an extract of infective larvae. [194]

Serological testing may represent a cost effective way to screen large populations for *S. stercoralis* infection; diagnostic sensitivity is usually high. Specificity is uncertain however; a positive result may represent cross-reactivity with non-*S. stercoralis* parasites, or serological persistence despite treatment and clearance. [195] Serological tests including an IgM component may have improved active strongyloidiasis detection, however supporting evidence is lacking currently. An analysis comparing five distinct serological tests for the detection of *S. stercoralis* found serology sensitivity to be 75.4-93.9% against a reference standard of *S. stercoralis* larvae detected in stool, and specificity 92.2-100% in individuals with no stool larvae and no exposure. [196] Four of the five serological tests showed varying degrees of cross reactivity in the presence of other parasitic disease.

3.1.5.1.1 *S. stercoralis* seroconversion after treatment

Whether serological responses revert to negative after successful *S. stercoralis* treatment is uncertain. Titers of antibodies against *S. stercoralis* may reduce after anti-helminthic therapy, however a serological cut-off for parasitological cure needs to be defined. [197] In a study of *S. stercoralis* seroconversion at least 3 months after receiving anti-helminthic therapy including at least one dose of ivermectin, 35 (83%) of 42 cases seroconverted from positive to negative, and 6 (14%) of 42 cases seroconverted from positive to equivocal. [198] Non-ivermectin based anti-helminthic therapies were less effective at achieving seroconversion. [198] In a further study of 40 individuals who had received at least two doses of ivermectin therapy, optical density (OD) was

used to measure reductions in antibody titers against *S. stercoralis*. [199] A fall in OD was noted in 38 (95%) of 40 individuals, and 26 (65%) of 40 individuals had reductions in OD consistent with cure.

3.1.5.2 *S. stercoralis* serology in HIV-infected individuals

The value of *S. stercoralis* serological tests in HIV co-infected individuals is unclear. Depletion of CD4+ T cells in advanced HIV disease leads to loss of humoral immunity which may reduce detectable antibody, and HIV-induced B cell hyperactivity may also create false positive serological results. [200] *S. stercoralis* ELISA sensitivities in HIV co-infection do not appear to match those seen in non-immunosuppressed individuals. [201] In a study of HIV co-infected immigrants to Italy, 15 (11%) of 138 individuals tested positive for *S. stercoralis*, however 4 cases positive by stool microscopy or stool culture had a negative serological test. [202]

3.1.5.3 *S. stercoralis* stool microscopy

Multiple diagnostic methods exist for *S. stercoralis* larvae detection in stool. Routine wet preparation stool microscopy has low sensitivity; larval shedding in stool is intermittent resulting in false negative cases. A stool concentration method increases the identification of ova, cysts and larvae, especially when parasite numbers are low. [203] The stool concentration method and alternative stool diagnostic methods to wet preparation microscopy are described in the appendix (B and C), respectively. A single stool examination may detect larvae in only 15-24% of positive cases, using direct faecal smear or formalin-ether concentration methods. [204]

3.1.5.4 *S. stercoralis* stool PCR

Stool PCR tests offer a potentially more sensitive approach to *S. stercoralis* identification. The stool excretion and distribution of parasite DNA is less variable than for parasite eggs, [205] however whilst PCR testing is more sensitive than stool microscopy, stool PCR remains insufficiently sensitive for *S. stercoralis* identification. A meta-analysis of 14 studies compared *S. stercoralis* stool PCR testing to conventional stool diagnostic methods and/or serology as a reference standard. [195] Stool PCR had high specificity, yet lacked sufficient diagnostic sensitivity (56.5% [95% CI 39.2-72.4%]) against a reference standard of stool larval detection and serology. [195]

3.1.6 Anti-helminthic therapy

3.1.6.1 Uncomplicated *S. stercoralis* infection

The optimal treatment of uncomplicated strongyloidiasis is ivermectin. Trial data have shown superiority of ivermectin over albendazole,[206–210] an improved side effect profile of ivermectin compared to thiabendazole,[180,211–213] and unlikely benefit of double dose ivermectin compared to single dose ivermectin.[214,215] Trial data assessing ivermectin, albendazole and thiabendazole for strongyloidiasis, and issues surrounding confirmation of cure, are described in appendix D.

3.1.6.2 Hyperinfection syndrome

Best therapy for *Strongyloides* hyperinfection is uncertain, and based on the life cycle of *S. stercoralis* rather than trial data. Anti-helminthic therapies may be ineffective on larvae that have not fully matured.[216] United States Center for Disease Control and Prevention (CDC) guidelines advise ivermectin 200µg/kg orally daily, with continuation of treatment until stool microscopy is negative for 2 weeks.[217] The action of ivermectin on extraintestinal parasites is unclear. In practice, daily ivermectin for a minimum of 2 weeks is commonplace,[191] however this duration may be too short to ensure all larval stages have matured to a therapy-susceptible stage. Immunosuppression is suspended if possible. Prophylaxis against *S. stercoralis* is discussed in appendix E. *S. stercoralis* co-infection in the context of necessary corticosteroid therapy lacks an evidence base to guide treatment.

3.1.7 Immunopathology of tuberculous meningitis

3.1.7.1 Pathogenesis of TBM

The current model of TBM disease follows a two-step process, with first a pulmonary focus, and then after *M. tuberculosis* dissemination through the blood stream, a CNS focus, which is the point of *M. tuberculosis* entry into the CSF.[9,15] Much of the damage caused in TBM is due to a dysregulated and excessive inflammatory response to *M. tuberculosis*. [9] The pathogenesis of TBM is described further in the introductory chapter (chapter 1) of this thesis.

3.1.7.2 Cerebrospinal fluid cytokine concentrations in tuberculous meningitis

Cytokines are messengers secreted by almost all cells, that act to control and alter their own behaviour or that of other cells, with a key role in modulating inflammation via a complex network of interactions.[218,219] In TBM pro-inflammatory cytokines are acutely elevated. In a study in India, CSF cytokine concentrations in 16 individuals with clinical TBM were compared with cytokine concentrations of 10 controls. Concentrations of TNF- α , IL-1 β , IL-6, IL-8, IL-10 were elevated in TBM, and declined during treatment, yet levels were not related to severity, brain MRI abnormalities nor clinical outcome.[25] In a separate study of 15 individuals with TBM (age range

12-58 years) CSF TNF- α , IL-10, and IFN- γ concentrations were persistently elevated, with elevation not related to stage or outcome.[26] In a paediatric study of TBM, CSF TNF- α , IL-1 β , and IFN- γ concentrations were elevated in acute TBM, however these concentrations did not correlate with disease severity, nor were they influenced by corticosteroid administration.[27]

In Vietnamese adults (age >14 years) recruited to a randomised double-blind placebo-controlled trial of dexamethasone for the treatment of TBM,[5] CSF cytokine concentrations (TNF, IL-1 β , IL-6, IL-8, IL-10, IL-12p70) were measured at multiple time points, and compared between patients receiving dexamethasone and placebo.[220] Dexamethasone did not significantly alter the levels of these CSF cytokines. CSF concentrations of IL-6, IL-8, and IL-10 fell slowly after commencement of anti-TB chemotherapy, and TNF fell rapidly, all irrespective of dexamethasone. IL-1 β , IL-12p70 were rarely detected in pre-treatment CSF samples (7/61 and 6/61 CSF samples, respectively). In a follow up study of patients from the same trial, low CSF IFN- γ was associated with death in adults (>14 years) with TBM and HIV co-infection.[221]

In a further study in Vietnamese adults with TBM, CSF concentrations of TNF- α , IFN- γ , IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-13 were measured.[18] In HIV uninfected adults with TBM concentrations of pro-inflammatory IL-1 β , IL-2, and IL-6 (but not TNF- α) were significantly associated with *LTA4H* genotype; low concentrations in CC genotype, intermediate concentrations in CT genotype, and high concentrations in TT genotype). *LTA4H* genotype did not affect CSF cytokine concentrations in HIV co-infected patients, in whom there was a global increase in pro-inflammatory cytokine concentrations.

3.1.8 *S. stercoralis* and immunomodulation

3.1.8.1 Pro-inflammatory vs. anti-inflammatory immunity

Two major types of effector T cell exist in humans; T helper and T cytotoxic, which display either CD4 or CD8 molecules respectively on their cell surface.[219] Effector Th1 and Th2 are distinct subpopulations of T lymphocytes expressing the CD4 receptor, and are differentiated by the cytokines they secrete.[219] A classical Th1 response is characterised by secretion of IFN- γ and IL-2, and activation of IL-12 secretion from macrophages, whereas a Th2 response is characterised by secretion of IL-4, IL-5, IL-6, IL-9, IL-10, IL-13 and IL-21.[219,222] Although some overlap exists in the variety of cytokines secreted by Th1 and Th2 cells, Th1 cells secrete IFN- γ but not IL-4, and Th2 cells secrete IL-4 but not IFN- γ . [223] A pattern of ‘type 1 immunity’ is largely regulated by Th1 cells, with a pro-inflammatory effect leading to, amongst other processes, phagocytosis and intracellular killing of microbes.[223] ‘Type 2 immunity’ involves stimulation of antibody by Th2

cells, an antibody class switch to IgE, IL-5 driven production and activation of eosinophils, and mast cell stimulation and degranulation.[223]

In humans, helminths, such as *S. stercoralis*, characteristically induce a host type 2 immune response, with activation of cells of the innate and adaptive immune systems, and marked elevation of IL-4, IL-5 and IL-13.[224] Nematode infection induces antigen-specific IgE production, which coats the nematode. Eosinophils bind to the antibody and release large cytotoxic granules onto the surface of the nematode to kill it.[219] Eosinophils have an additional role as an antigen presenting cell in defense against *S. stercoralis*, activating T cells via major histocompatibility complex class II.[225]

Interplay between Th1 and Th2 pathways are complex. The ‘hygiene hypothesis’ suggests exposure to bacterial and viral infections in early life may influence future immune responses to allergens, as impairment of the development of type 1 immunity results in imbalance in immunity and excessive type 2 immune responses to allergens.[226,227] Similarly, a lack of helminth exposure during childhood and throughout life may result in a dysregulated immune system and an increased risk of atopic and autoimmune disease.[222] Th1 and Th2 responses appear cross-inhibitory, with an increase in either Th1 or Th2 cells coupled with a reduction in the other.[228,229] Helminth co-infection, and the Th2 immune response this produces may either beneficially reduce pathology, or impair essential lifesaving immune responses.[226]

The ability of Th2 cells to downregulate Th1 processes raises questions regarding host immune function in the context of bacterial infection and helminth co-infection.[224] Helminth co-infection appears to modulate the host immune response in the context of *M. tuberculosis* infection, with impairment of IFN- γ response and suppression of type 1 immune responses, resulting in more severe TB disease.[224]

3.1.8.2 *S. stercoralis* co-infection and tuberculosis

In a study of 40 individuals with pulmonary TB, helminth co-infection was diagnosed in 11 (28%) of 40 individuals (in 8/11 cases the helminth was *S. stercoralis*).[230] A non-significant trend was found towards more severe disease in helminth co-infected individual with pulmonary TB vs. the TB-only control group. Interestingly, there were significantly lower blood IFN- γ levels in helminth co-infected individuals vs. the TB-only control group, both at presentation and at completion of 24 weeks of anti-TB chemotherapy.[230] This study suggests suppression of Th1 responses required for effective TB control in helminth co-infection.[230] A larger study of plasma inflammatory cytokines in individuals with pulmonary TB (n=88, 42/88 co-infected with *S. stercoralis*) and latent

TB (n=88, 44/88 co-infected with *S. stercoralis*) found significantly lower levels of TNF- α , IFN- γ and IL-2, (key cytokines involved in type 1 immunity and the immune response to TB), in pulmonary TB patients co-infected with *S. stercoralis* compared to in the TB control group.[53] In addition plasma concentrations of type 2 and regulatory cytokines (IL-4, IL-5, IL-10 and IL-13) were significantly elevated in individuals with latent TB and *S. stercoralis* (n=44) vs. individuals with only latent TB (n=44). Interestingly a significant difference in these cytokine concentrations was not seen in active TB with and without *S. stercoralis* infection.

3.1.9 Research objectives

Whether such an immunomodulatory role is seen with *S. stercoralis* co-infection in TBM is unknown. Given mortality and morbidity of TBM are often through an excessive host inflammatory response, suppression of Th1 responses in TBM have potential to be neuroprotective.

Here I will describe the frequency of *S. stercoralis* co-infection in TBM. I will investigate the influence of *S. stercoralis* on TBM clinical presentation, on the intracerebral inflammatory response to *M. tuberculosis* through routine CSF parameters and CSF cytokine analysis, and on outcomes of TBM.

3.2 Methods

3.2.1 Study participants

Participants were enrolled from two on-going randomised placebo-controlled phase III trials of adjunctive corticosteroid therapy for HIV co-infected and uninfected adults with TBM (ACT HIV; [clinicaltrials.gov NCT03092817](https://clinicaltrials.gov/ct2/show/study/NCT03092817)[85] or LAST ACT; [clinicaltrials.gov NCT03100786](https://clinicaltrials.gov/ct2/show/study/NCT03100786)[41]).

Participants in this study of *S. stercoralis* co-infection in TBM are Vietnamese adults (≥ 18 years of age), with or without HIV co-infection, with a clinical diagnosis of TBM (symptoms and CSF abnormalities consistent with TBM, and anti-TB chemotherapy planned or started by the attending clinician). Potential participants were excluded if an additional brain infection to TBM was suspected, more than 6 consecutive days of anti-TB chemotherapy were received, more than 3 days (ACT HIV, and LAST ACT until September 2018) or more than 6 days (LAST ACT after September 2018) of corticosteroid were received, dexamethasone was mandatory or contraindicated, or the patient had already entered the ACT HIV or LAST ACT trial. Eligible participants were enrolled into ACT HIV or LAST ACT between June 2017 and December 2019, inclusive, at HTD or Pham Ngoc Thach Hospital for Tuberculosis and Lung Disease (both in Ho Chi Minh City, Vietnam).

All enrolled participants (or a family member if the patient was incapacitated) gave written informed consent to participate in this study. Ethical approvals for ACT HIV and LAST ACT were obtained from OxTREC (36-16 and 52-16, respectively), the ethical committees of HTD (14/ HDDD and 37/ HDDD, respectively) and Pham Ngoc Thach Hospital for Tuberculosis and Lung Disease (1033/ HDDD-PNT and 460/ HDDD-PNT, respectively), and from the Vietnam Ministry of Health (108/CN-BDGDD and 151/CN-BDGDD, respectively). ACT HIV and LAST ACT are funded by an Investigator Award to Professor Guy Thwaites (Wellcome Trust, UK: 110179/Z/15/Z).

3.2.2 Clinical data

Demographic data (age, gender), baseline MRC TBM severity grade[9] and HIV co-infection status were recorded. Death and neurological complications by 3 months were recorded. Neurological complications were defined as any of a fall in GCS of ≥ 2 points for ≥ 48 hours, a focal neurological sign, seizure, cerebellar signs, coma, or cerebral herniation.

3.2.3 *S. stercoralis* testing

3.2.3.1 Schedule

All enrolled participants were tested for *S. stercoralis*. *S. stercoralis* serology (NovaTec Immunodiagnostica GmbH, Dietzenbach, Germany) was performed in all participants at baseline (defined as day of signing informed consent). Stool microscopy was performed in each participant at baseline, and again at day 21-30 prior to discharge if the participant was discharged home (to ensure eradication of larvae). Stool *S. stercoralis* PCR testing was performed in a sub-group of participants (those with *S. stercoralis* infection diagnosed by serology or stool microscopy [allowing comparison of diagnostic tests], and in consecutively enrolled participants negative for *S. stercoralis* until approximately 200 samples had been identified for stool *S. stercoralis* PCR testing). A limit was placed on PCR testing due to test cost. An eosinophil count (full blood count) was tested at baseline.

3.2.3.2 Serology

S. stercoralis serology (an immunoenzymatic determination of IgG and IgM antibodies against *S. stercoralis*) was performed following manufacturer instructions. As per manufacturer (NovaTec Immunodiagnostica GMBH) details; microtiter strip wells are pre-coated with recombinant *S. stercoralis* antigens to bind corresponding antibodies of the specimen, unbound sample material is removed by washing the wells, and bound *S. stercoralis* specific antibodies are detected by the

additional of horseradish peroxidase labeled anti-human IgG and IgM conjugate. The subsequently formed immune complex is visualized by adding tetramethylbenzidine substrate which gives a blue reaction. The intensity is proportional to the amount of *S. stercoralis*-specific antibodies in the specimen. The addition of sulphuric acid stops the reaction. Absorbance at 450nm is read using an ELISA microwell plate reader. Serology results were reported as follows; positive: >11 units, indicating antibodies against the pathogen are present; equivocal: 9-11 units, indicating antibodies against the pathogen could not be detected clearly, recommend repeat in 2-4 weeks (a repeat equivocal test means a negative result); negative: <9 units, indicating the sample contains no antibodies against the pathogen and the test is negative. Product specifications report 87.9% sensitivity and 95.8% specificity for this test.

3.2.3.3 Stool PCR

Routine wet preparation microscopy was used to identify *S. stercoralis* larvae. Stool concentration methods were not used. Stool *Strongyloides stercoralis* PCR was performed using an in-house (OUCRU, Ho Chi Minh City, Vietnam) stool PCR developed from the materials and methods of Verweij et al[205] (a real time PCR assay targeting the 18S ribosomal RNA gene sequence of *S. stercoralis*). Subsequently tested against 212 stool samples from Ghana and compared against the Baermann stool method and two stool microscopies for the detection of L1 or L3 *S. stercoralis* larvae, this stool PCR [205] showed 61% sensitivity (33/54 cases positive) for *S. stercoralis* detection. Stool PCR was positive in 12 (7.6%) of 158 samples negative by conventional tests, and excellent stool PCR specificity was demonstrated when the stool PCR was tested against stool 'controls'.

3.2.4 Cerebrospinal fluid testing

3.2.4.1 Tuberculosis

At least 6mls of lumbar CSF was sampled (if available) at baseline in all participants. All CSF volumes were recorded. CSF processing and testing followed previously described procedures.[37] CSF supernatant was removed and stored at -80°C for future CSF cytokine testing.

3.2.4.2 Cytokines

Cytokines were selected for CSF concentration analysis based on previous CSF cytokine studies in TBM as previously described, cytokines predicted to be affected by *S. stercoralis* co-infection,[53] and availability of testing kits (RnD Systems, Inc, Minnesota, USA); CSF TNF- α , IFN- γ , IL-1 β , IL-2, IL-4, IL-5, IL-6, IL10, IL-12p70 and IL-13 were measured (table 3-1).

Table 3-1: CSF cytokines, site of production, and role in immunity

Cytokine	Produced by	Role
TNF- α	Macrophages	Immune activation and induction of an inflammatory response. Pro-inflammatory.[231]
IFN- γ	T cells and NK cells	Activates macrophage and neutrophil intracellular killing, stimulates NK function, increases MHC class II expression on APCs. Antiviral actions. Pro-inflammatory.[231]
IL-1 β (IL-1 family)	Macrophages, B cells, dendritic cells	Role in innate immunity and inflammation. Pro-inflammatory.[231,232]
IL-2	Mostly T cells	Activates CD4+ T cells, CD8+ T cells, and NK cells, supporting their growth and function. Involvement in adaptive immunity. Mostly pro-inflammatory.[233]
IL-4	CD4+ T cells	B and T lymphocyte proliferation, enhancement of MHC II expression, involvement in IgE and IgG responses. Anti-inflammatory.
IL-5	CD4+ T cells	Promotes growth of B cells and eosinophils, stimulates IgA and IgM production. Anti-inflammatory.
IL-6	CD4+ T cells, macrophages, fibroblasts	Promotes B cell growth and IgG production, induces acute phase responses. Pro-inflammatory.[231]
IL-10	CD4+ T cells, activated monocytes	Inhibits the production of IFN- α , IL-1, IL-6, TNF- α , and stops antigen presentation. Anti-inflammatory.
IL-12p70 (p35/p40 subunits of IL-12)	T cells	Induces IFN-gamma production and supports T cell responses. Mostly pro-inflammatory.[234]
IL-13	Activated T cells	Stimulates B cells. Anti-inflammatory.

Table based on data from Parkin and Cohen[219] (with original reference for table Kumar and Clark, Clinical Medicine, 4th edition) and Turner et al.[218] Additional data referenced in the table. APC=antigen presenting cell. CD=cluster of differentiation. IL=interleukin. MHC=major histocompatibility complex. NK=natural killer.

CSF cytokine testing was performed by magnetic microbead immunoassay (RnD Systems, Inc, Minnesota, USA) following manufacturer instructions.[235] Briefly, magnetic microparticles coated with cytokine-specific antibodies were combined with CSF samples in a 96-well plate. Addition of Streptavidin-phycoerythrin after washing phases allows light emitting diodes (LEDs) to quantify the cytokine bound to the microparticle, whereas another LED identifies the microparticle region (and thus identifies the cytokine). CSF samples underwent cytokine concentration measurement across 2 plates, alongside 6 sequentially diluted standards on each plate. Cytokine concentrations were measured using a Luminex 200 (Luminex, Texas, USA). Data analysis set up of the Luminex 200 stated use of a 50µL CSF sample, however in practice 180µL of CSF was used. Therefore, final results were divided by 3.6 (180/50). Cytokine concentrations were measured using a Luminex 200 (Luminex, Texas, USA). The Luminex 200 xPONENT version 3.1.971.0 was used for analysis.

3.2.4.2.1 Derivation of undetected cytokine concentrations

Where CSF cytokine concentrations were undetected, either the lowest limit of extrapolation divided by 2, or the lowest limit of detection divided by two, was used, whichever was lowest. CSF cytokine testing was performed across two 96-well plates, and value extrapolation was plate-specific. Rarely, where cytokine concentrations were too high for quantification, the upper limit of detection multiplied by 2 was used; samples and testing kits were unavailable for sample dilution and repeat testing. Log2 calculations of CSF cytokine concentration were performed.

3.2.5 Treatment

3.2.5.1 Anti-tuberculous chemotherapy

All participants received anti-TB chemotherapy following Vietnamese national guidelines. Rifampicin (10mg/kg/24 hours; maximum dose 600mg), isoniazid (5mg/kg/24 hours; maximum 300mg), pyrazinamide (25mg/kg/24 hours; maximum 2g), and ethambutol (20mg/kg/24 hours; maximum 1.2g), were given as outlined in the published study protocols.[41,85] Total anti-TB chemotherapy duration is 12 months, with pyrazinamide discontinued after 2 months. In cases of drug resistance, or where adverse events necessitate treatment discontinuation, local and national advice will be followed. Drug regimens are further described in appendix F.

3.2.5.2 Study drug for ACT HIV and LAST ACT

Additionally, all participants were randomised to dexamethasone or placebo (termed ‘study drug’), a double blinded allocation following 1:1 randomisation (except *LTA4H* TT-genotype HIV uninfected participants from LAST ACT [~7% total participants] who all received open-label dexamethasone). Study drug was administered over 8 weeks (TBM grade 2 or 3) or 6 weeks (TBM grade 1), following a tapering course, with weekly reductions (table 3-2). The ACT HIV and LAST ACT trials are ongoing and treatment allocations remain blinded.

Table 3-2: Study drug doses in ACT and LAST ACT after randomisation[41,85]

	MRC Grade I Daily dexamethasone dose/route	MRC Grades II and III Daily dexamethasone dose/route
Week 1	0.3 mg/kg/24 hrs IV	0.4 mg/kg/24 hrs IV
Week 2	0.2 mg/kg/24 hrs IV	0.3 mg/kg/24 hrs IV
Week 3	0.1 mg/kg/24 hrs IV	0.2 mg/kg/24 hrs IV
Week 4	3mg/24 hrs oral	0.1 mg/kg/24 hrs IV
Week 5	2mg/24 hrs oral	4 mg/24 hrs oral
Week 6	1 mg/24 hrs oral	3 mg/24 hrs oral
Week 7	Stop	2 mg/24 hrs oral
Week 8		1 mg/24 hrs oral

IV=intravenous. MRC=Medical Research Council

3.2.5.3 *S. stercoralis*

Treatment of *S. stercoralis* follows Vietnamese local and national guidelines. Uncomplicated *S. stercoralis* infection is treated with ivermectin 200µg/kg daily for 1-2 days. All stool positive cases receive *S. stercoralis* eradication therapy. Participants only positive by serology are treated on a case-by-case basis, with clinical state, risks for dissemination, and magnitude of serological titer, considered. Complicated *S. stercoralis* infection is treated with ivermectin for at least 2 weeks, and repeat negative stool samples are then sought.

3.2.6 Statistical analysis

3.2.6.1 Sample size

The prevalence of *S. stercoralis* co-infection in Vietnamese adults with TBM is not known. A prevalence from 7.4-18.2% may be expected using serological estimates from non-TBM cohorts.[186,187] In the study by George et al,[53] plasma cytokines were measured in 42 *S. stercoralis* co-infected individuals with pulmonary TB, and significant differences in plasma cytokine concentrations were found vs. a non-*S. stercoralis* group. However, given immunomodulation in TBM by *S. stercoralis* is uncertain, a sample size calculation was not

possible and this study was exploratory. CSF cytokine concentration analysis was based upon sample availability.

3.2.6.2 Analysis populations

Primary analysis populations were selected based on clinical categories of *S. stercoralis* infection. A *S. stercoralis* ‘uninfected’ group was selected based upon participants having been tested by all of *S. stercoralis* serology, stool microscopy, and stool PCR, and all being negative. This approach gave the highest certainty of a *S. stercoralis* uninfected status. A ‘past infection’ group was selected based upon participants having positive *S. stercoralis* serology with no positive stool result (but at least one of stool microscopy or stool PCR performed). An ‘active infection’ group was selected based upon a positive *S. stercoralis* stool microscopy or stool PCR result, regardless of other testing performed.

In addition, secondary analyses were performed on three additional sub-populations; participants who had serology performed, participants who had both serology and stool microscopy performed (divided into groups A-C), and participants who had all of serology, stool microscopy and stool PCR performed (divided into groups 1-4) (figure 3-1). These secondary analyses compared baseline TBM severity, CSF inflammatory parameters, and clinical endpoints (neurological complications by 3 months, and death by 3 months) between those with and without positive *S. stercoralis* tests, for each sub-population. CSF cytokine analysis was performed only for primary analysis populations.

3.2.6.3 Statistical tests

Comparison between proportions was assessed by the chi squared test. Non-normally distributed variables, including CSF cytokine concentrations, were compared using the Wilcoxon rank sum test. Given variable CSF concentrations between cytokines, ratios of cytokine changes were compared and tabulated. A multivariate analysis (with odds ratios and 95% CIs) was performed to evaluate whether age, MRC TBM grade, HIV co-infection and active *S. stercoralis* infection predicted neurological complications by 3 months. Data were analysed using R (version 3.6).

3.2.6.4 Statistical analysis plan

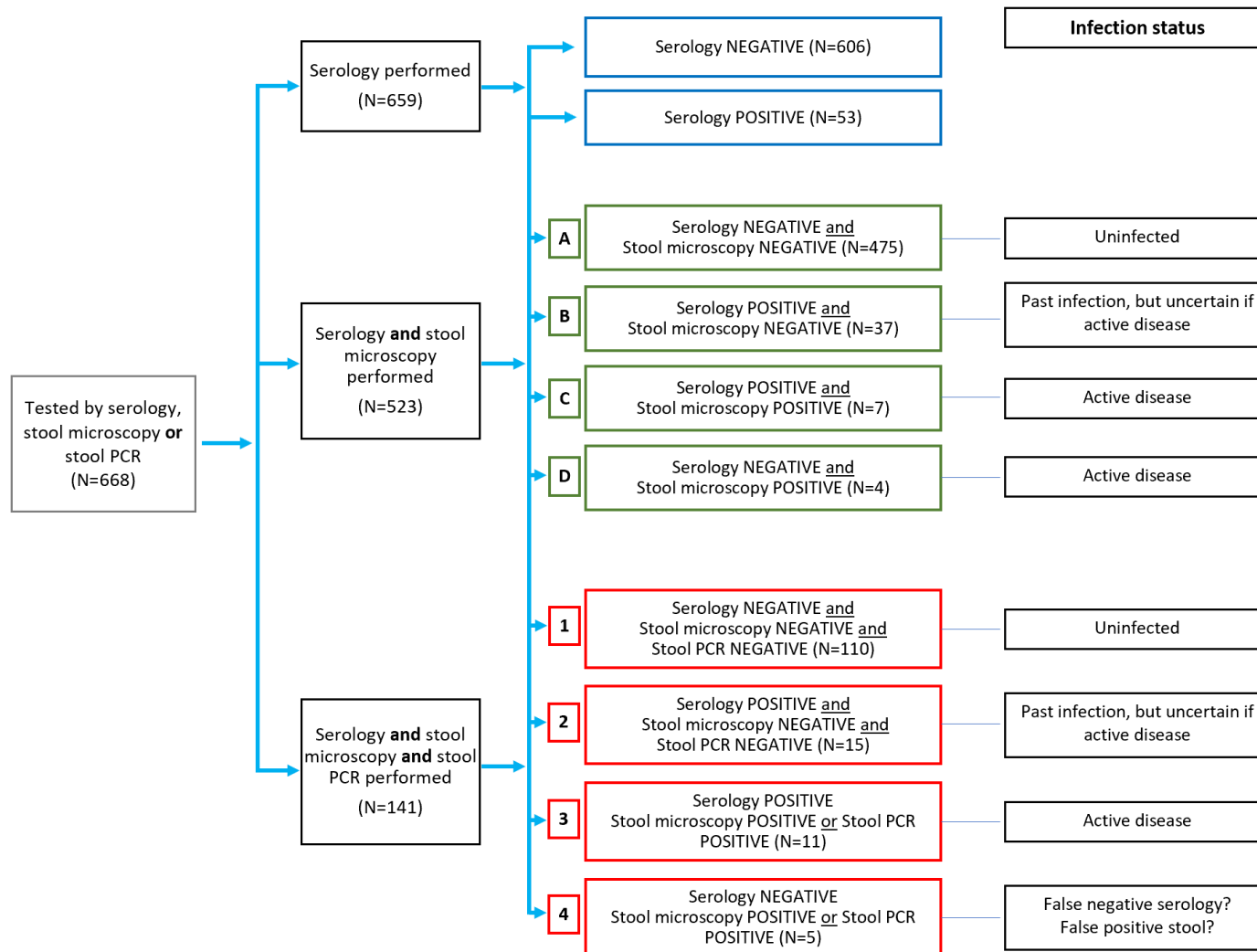
A statistical analysis plan written in advance of data analysis outlined the following:

1. Evaluate the number of positive *S. stercoralis* serological, stool microscopy, and stool PCR tests, and compare by age, sex, and HIV co-infection

2. Compare HIV co-infection rate, TBM final diagnosis, MRC TBM grade, and baseline blood eosinophil count between participants uninfected for *S. stercoralis*, and participants with active or past *S. stercoralis* co-infection
3. Compare CSF TNF- α , IFN- γ , IL-1 β , IL-2, IL-4, IL-5, IL-6, IL10, IL-12p70 and IL-13 between participants with and without *S. stercoralis* co-infection
4. Compare neurological complications by 3 months, and death by 3 months, between participants uninfected for *S. stercoralis*, and participants with active or past *S. stercoralis* co-infection

Subsequent to the initial data analysis, given the immunomodulation suggested by clinical presentation, CSF cytokine, and clinical endpoint data, routine CSF parameters (WBC, neutrophil count, neutrophil percentage, and protein), CSF/blood glucose ratio, and Xpert positivity were also compared between participants with and without *S. stercoralis* co-infection to further evaluate the effect of *S. stercoralis* on CSF inflammation.

Figure 3-1: Populations stratified by *S. stercoralis* tests performed



N = number of participants

3.3 Results

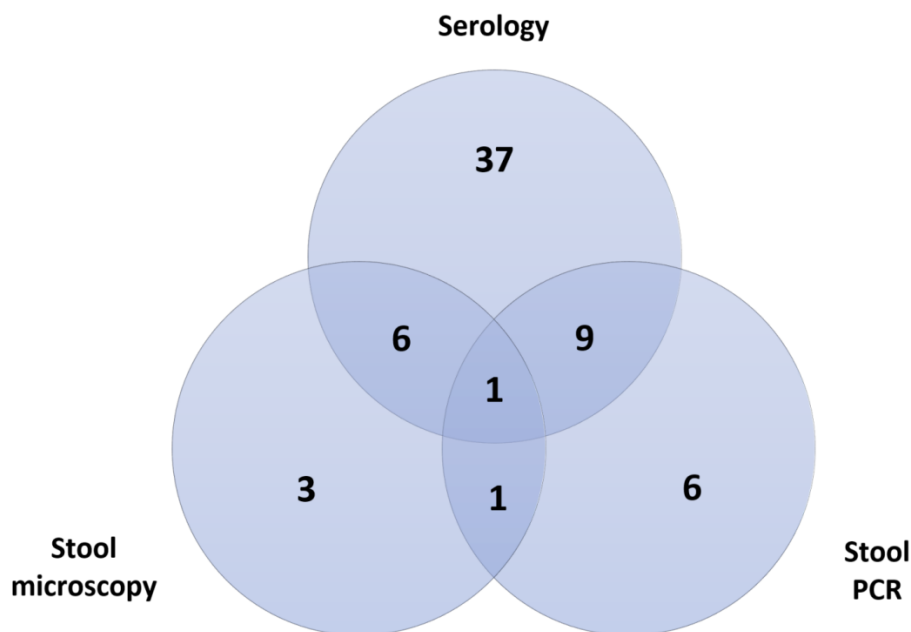
3.3.1 The study population

From June 2017 to December 2019 inclusive, 668 participants with clinical TBM underwent baseline testing for *S. stercoralis* co-infection, by one or more of serology, stool microscopy, and stool PCR. The median age of the study population was 39 (IQR 31-50) years. 67.5% (451/668) participants were male. MRC TBM severity grades[9,236] amongst the study population were, Grade 1: 45.1% (n=301), Grade 2: 43.3% (n=289), Grade 3: 11.7% (n=78). 43.4% (n=290) study participants were diagnosed with definite TBM, 38.3% (n=256) with probable TBM, and 16.0% (n=107) with possible TBM. 44.6% (298/668) participants were HIV co-infected.

3.3.2 *S. stercoralis* testing

Overall, 9.4% (63/668) participants tested positive for *S. stercoralis* by at least one of serology (n=53), stool microscopy (n=11), or stool PCR (n=17). All three diagnostic tests were performed in 141/668 (21.1%) participants, with a positive result for all three tests in only one participant (figure 3-2).

Figure 3-2: Venn diagram of positive *S. stercoralis* tests



Positive tests: 53 serology, 11 stool microscopy, 17 stool PCR. 81 positive *S. stercoralis* tests represented in 63 patients testing positive for *S. stercoralis* by serology, stool microscopy, stool PCR or combinations of these tests. PCR=polymerase chain reaction

A positive *S. stercoralis* diagnosis was made by stool microscopy alone in 3 participants and by stool PCR alone in 6 participants. The total numbers of *S. stercoralis* tests performed are shown in figure 3-1. The median age of participants who tested positive for *S. stercoralis* by any method was 49 (IQR 37-59) years vs. 40 (IQR 32-51) years in participants who tested negative for *S. stercoralis* by serology, stool microscopy, and stool PCR. 55/63 (87.3%) of *S. stercoralis* positive participants were male vs. 75/110 (68.2%) of *S. stercoralis* negative participants. HIV co-infection was present in 16/63 (25.4%) *S. stercoralis* positive participants vs. in 37/110 (33.6%) *S. stercoralis* negative participants.

3.3.3 Influence of *S. stercoralis* infection on TBM presentation and routine CSF parameters

A comparison of baseline TBM severity and routine CSF parameters between primary analysis populations is shown in table 3-3.

Table 3-3: A comparison of baseline TBM severity and CSF inflammatory parameters for cytokine testing groups

	<i>S. stercoralis</i> testing				
	Uninfected	Past infection	P value	Active infection	P value
Patients (No.)	110	30		26	
HIV status (No. [%])					
- Positive	37 (33.6%)	4 (13.3%)	0.05	9 (34.6%)	1.0
- Negative	73 (66.4%)	26 (86.7%)		17 (65.4%)	
Final diagnosis					
- Definite	58 (52.7%)	9 (30.0%)	Ref	5 (19.2%)	Ref
- Probable	38 (34.5%)	14 (46.7%)	0.11	16 (61.5%)	0.01
- Possible	13 (11.8%)	6 (20.0%)	0.13	5 (19.2%)	0.06
MRC TBM Grade (No. [%])					
- 1	44 (40.0%)	16 (53.3%)	Ref	13 (50.0%)	Ref
- 2	45 (40.9%)	12 (40.0%)	0.62	12 (46.2%)	1.0
- 3	21 (19.1%)	2 (6.7%)	0.14	1 (3.8%)	0.11
Baseline eosinophil count (10 ⁹ /L) (Median[IQR])	0 (0-0.1)	0.2 (0.1-0.2)	<0.001	0.1 (0-0.4)	0.02
CSF WBC (cells/mm ³) (Median[IQR])	123 (29-297)	74 (9-254)	0.20	70 (7-168)	0.13
CSF neutrophil count (cells/mm ³) (Median[IQR])	14 (1-83)	6 (0-36)	0.25	3 (0-25)	0.04

CSF neutrophil percentage (Median[IQR])	10 (5-27)	11 (0-15)	0.20	5 (0-14)	0.04
CSF/blood glucose ratio (Median[IQR])	0.38 (0.26-0.52)	0.43 (0.33-0.52)	0.34	0.45 (0.31-0.59)	0.16
CSF protein (g/L) (Median[IQR])	1.45 (0.95-2.18)	1.39 (1.13-1.94)	0.69	0.94 (0.60-1.84)	0.08
Xpert (No. [%])					
- Positive	31 (28.2%)	6 (20.0%)	0.50	1 (3.8%)	0.03
- Negative	68 (61.8%)	21 (70.0%)		24 (92.3%)	

P values are shown for group comparison with *S. stercoralis* uninfected group in each case. The chi squared test was used to compare categorical data. Ref: with final diagnosis, P values represent comparison of each of probable and possible TBM, with definite TBM. With MRC TBM Grade P values represent comparison of each of Grade 2 and Grade 3 TBM, with Grade 1 TBM. With final diagnosis, n=1 (past infection group) participant scored < 6 points for the TBM diagnostic score.[69] This case was considered to be TBM by the treating clinician and was treated as such. The Wilcoxon rank sum test was used to compare continuous data. Uninfected = all 3 testing methods used, and all negative (correlates with group '1' in figure 3-1). Past infection = positive *S. stercoralis* serology with no positive stool testing (but at least one of stool microscopy or stool PCR performed). Active infection = Positive stool microscopy or stool PCR for *S. stercoralis*, regardless of other testing performed. Baseline eosinophil counts, CSF WBC, and CSF neutrophil percentage are non-normally distributed and are shown as median (IQR). 'Ref' refers to the final diagnosis or grade against which comparison was made. CSF=cerebrospinal fluid. HIV=human immunodeficiency virus. IQR=interquartile range. MRC=Modified Research Council. TBM=tuberculous meningitis. WBC=white blood cell. Xpert=GeneXpert MTB/RIF

Baseline eosinophils were significantly elevated in active *S. stercoralis* infection compared with *S. stercoralis* uninfected participants; $0.1 \times 10^9/L$ (0-0.4) vs. $0 \times 10^9/L$ (0-0.1) respectively, $p=0.02$. Median CSF neutrophil count and neutrophil percentage were reduced in active *S. stercoralis* infection compared with uninfected participants; 3 cells/mm³ (0-25) vs. 14 cells/mm³ (1-83) respectively, $p=0.04$, and 5% (0-14) vs. 10% (5-27) respectively, $p=0.04$. Additionally, trends towards reduced grade 3 disease (3.8% [1/26] vs. 19.1% [21/110]), reduced total CSF WCC cells/mm³ (70 [7-168] vs. 123 cells/mm³ [29-297]), reduced CSF protein (0.94g/L [0.60-1.84] vs. 1.45g/L [0.95-2.18]), and elevated CSF/blood glucose ratio (0.45 [0.31-0.59] vs. 0.38 [0.26-0.52]), were seen with active *S. stercoralis* infection vs. uninfected participants, respectively. In active *S.*

stercoralis infection there was a reduced proportion of definite TBM; 19.2% (5/26) vs. 52.7% (58/110) ($p=0.01$ vs. probable TBM), and reduced Xpert positivity; 3.8% (1/26) vs. 28.2% (31/110) ($p=0.03$), compared with uninfected participants.

Baseline TBM severity and CSF inflammatory parameter analyses performed in sub-populations of participants who had serology performed, or both serology and stool microscopy performed, are shown in tables 3-4 and 3-5.

Table 3-4: A comparison of baseline TBM severity and CSF inflammatory parameters in participants who had *S. stercoralis* serology performed

	<i>S. stercoralis</i> testing		
	Negative <i>S. stercoralis</i> serology	Positive <i>S. stercoralis</i> serology	P value
Patients (No.)	606	53	
HIV status (No. [%])			
- Positive	280 (46.2%)	11 (20.8%)	0.001
- Negative	326 (53.8%)	42 (79.2%)	
Final diagnosis			
- Definite	272 (41.2%)	16 (30.2%)	Ref
- Probable	223 (39.4%)	26 (49.1%)	0.05
- Possible	97 (17.3%)	10 (18.9%)	0.26
MRC TBM Grade (No. [%])			
- 1	267 (44.1%)	28 (52.8%)	Ref
- 2	266 (43.9%)	20 (37.7%)	0.35
- 3	73 (12.0%)	5 (9.4%)	0.53
Baseline eosinophil count (10 ⁹ /L)	0	0.1	0.002
(Median[IQR])	(0-0.1)	(0-0.3)	
CSF WBC (cells/mm ³)	75	70	0.90
(Median[IQR])	(6-243)	(7-257)	
CSF neutrophil count (cells/mm ³)	6	4	0.86
(Median[IQR])	(0-46)	(0-49)	
CSF neutrophil percentage	8	10	0.95
(Median[IQR])	(0-22)	(0-15)	
CSF/blood glucose ratio	0.39	0.39	0.51

(Median[IQR])	(0.26-0.50)	(0.29-0.51)	
CSF protein (g/L)	1.38	1.46	0.36
(Median[IQR])	(0.73-2.19)	(0.91-2.24)	
Xpert (No. [%])			
- Positive	161 (26.6%)	10 (18.9%)	0.25
- Negative	410 (67.7%)	41 (77.4%)	

‘Negative *S. stercoralis* serology group corresponds with ‘serology NEGATIVE’ group in figure 3-1. ‘Positive *S. stercoralis* serology group corresponds with ‘serology POSITIVE’ group in figure 3-1. With final diagnosis, n=2 (negative *S. stercoralis* serology) and n=1 (positive *S. stercoralis* serology) participants scored < 6 points for the TBM diagnostic score.[69]. These were considered to be TBM by the treating clinician and were treated as such. With final diagnosis, P values represent comparison of each of probable and possible TBM, with definite TBM. With MRC TBM Grade P values represent comparison of each of Grade 2 and Grade 3 TBM, with Grade 1 TBM. The chi squared test was used to compare categorical data. The Wilcoxon rank sum test was used to compare continuous data. Baseline eosinophil counts, CSF WBC, and CSF neutrophil percentage are non-normally distributed and are shown as median (IQR). CSF=cerebrospinal fluid. HIV=human immunodeficiency virus. IQR=interquartile range. MRC=Modified Research Council. TBM=tuberculous meningitis. WBC=white blood cell. Xpert=GeneXpert MTB/RIF. ‘Ref’ refers to the final diagnosis or grade against which comparison was made.

Table 3-5: A comparison of baseline TBM severity and CSF inflammatory parameters in participants who had *S. stercoralis* serology and stool microscopy performed

	<i>S. stercoralis</i> testing				
	Group A	Group B		Group C	
	Negative by serology and stool microscopy	Positive by serology and negative by stool microscopy	P value	Positive by both serology and by stool microscopy	P value
Patients (No.)	475	37		7	
HIV status (No (%))					
- Positive	199 (41.9%)	7 (18.9%)	0.01	1 (14.3%)	0.29
- Negative	278 (58.5%)	30 (81.1%)		6 (85.7%)	
Final diagnosis					
- Definite	208 (43.8%)	9 (24.3%)	Ref	2 (28.6%)	Ref
- Probable	172 (36.2%)	19 (51.4%)	0.03	3 (42.9%)	0.84
- Possible	83 (17.5%)	8 (21.6%)	0.18	2 (28.6%)	0.70
MRC TBM Grade (No (%))					
- 1	220 (46.3%)	21 (56.8%)	Ref	5 (71.4%)	Ref
- 2	202 (42.5%)	15 (40.5%)	0.59	2 (28.6%)	0.53
- 3	53 (11.2%)	1 (2.7%)	0.15	0 (0%)	0.60
Baseline eosinophil count (10 ⁹ /L) (Median[IQR])	0.1 (0-0.2)	0.2 (0.1-0.3)	0.002	0.1 (0-0.6)	0.30
CSF WBC (cells/mm ³) (Median[IQR])	72 (8-253)	70 (6-243)	0.55	72 (49-285)	0.56

CSF neutrophil count (cells/mm ³) (Median[IQR])	5 (0-46)	4 (0-35)	0.81	3 (2-43)	0.89
CSF neutrophil percentage (Median[IQR])	5 (0-20)	5 (0-15)	0.85	8 (3-13)	0.79
CSF/blood glucose ratio (Median[IQR])	0.39 (0.26-0.50)	0.46 (0.35-0.53)	0.06	0.34 (0.26-0.46)	0.81
CSF protein (g/L) (Median[IQR])	1.35 (0.75-2.17)	1.29 (0.75-1.71)	0.36	1.49 (0.94-1.87)	0.78
Xpert					
- Positive	125 (26.3%)	6 (16.2%)	0.24	1 (14.3%)	0.71
- Negative	323 (68.0%)	29 (78.4%)		6 (85.7%)	

Groups A, B and C correspond with testing groups A, B and C in figure 3-1. With final diagnosis, n=1 (negative for *S. stercoralis* by serology and stool microscopy) and n=1 (positive for *S. stercoralis* by serology and negative by stool microscopy) participants scored < 6 points for the TBM diagnostic score.[69]. These were considered to be TBM by the treating clinician and were treated as such. With final diagnosis, P values represent comparison of each of probable and possible TBM, with definite TBM. With MRC TBM Grade P values represent comparison of each of Grade 2 and Grade 3 TBM, with Grade 1 TBM. P values are shown for comparison with negative group in each case. The chi squared test was used to compare categorical data. The Wilcoxon rank sum test was used to compare continuous data. Baseline eosinophil counts, CSF WBC, and CSF neutrophil percentage are non-normally distributed and are shown as median (IQR). CSF=cerebrospinal fluid. HIV=human immunodeficiency virus. IQR=interquartile range. MRC=Modified Research Council. TBM=tuberculous meningitis. WBC=white blood cells. Xpert=GeneXpert MTB/RIF. 'Ref' refers to the final diagnosis or grade against which comparison was made.

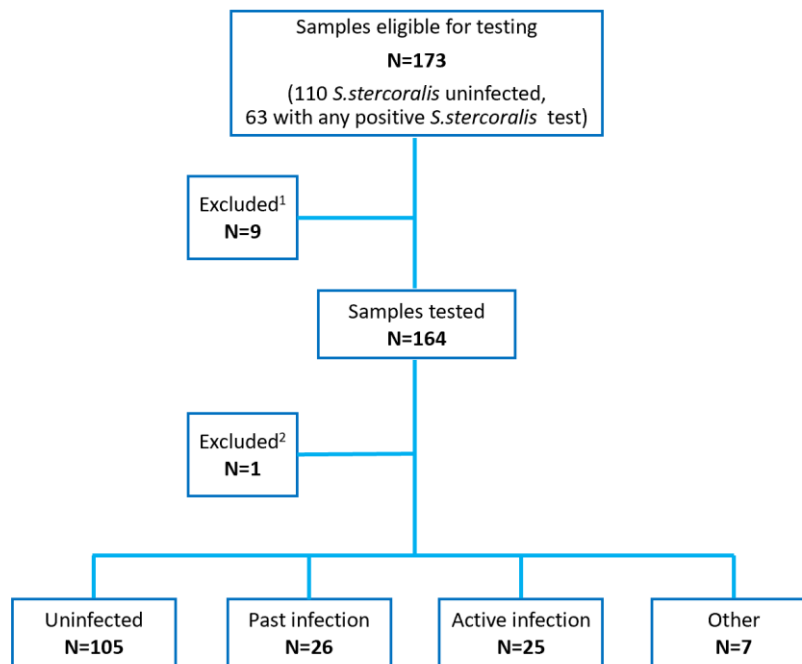
In participants who had *S. stercoralis* serology performed, a significantly reduced proportion of HIV co-infection was found in the positive *S. stercoralis* serology group compared with the negative *S. stercoralis* serology group (20.8% [11/53] vs. 46.2% [280/606], respectively, p=0.001) (table 3-4). In participants tested by both *S. stercoralis* serology and stool microscopy, a reduction in definite TBM (vs. probable TBM) was seen in participants with positive *S. stercoralis* serology

and negative stool microscopy vs. in participants testing negative by both *S. stercoralis* serology and stool microscopy (24.3% [9/37] vs. 41.2% [208/475], $p=0.03$) (table 3-5).

3.3.4 Baseline CSF cytokine concentrations in *Strongyloides stercoralis* co-infection

I hypothesised that in those with active *S. stercoralis* infection CSF concentrations of the pro-inflammatory cytokines IFN- γ , IL-2, and TNF- α would be reduced, and CSF concentrations of the regulatory cytokines IL-4, IL-5, IL-10, and IL-13 would be increased, compared with *S. stercoralis* uninfected participants. These cytokines, in addition to IL-1 β , IL-6 and IL-12p70 (as exploratory analyses) were measured in CSF, and compared between primary analysis populations. CSF cytokine testing flow is shown in figure 3-3.

Figure 3-3: CSF cytokine testing flow diagram



14 samples were excluded from testing when no stored CSF sample was available. 5 samples were not tested as *S. stercoralis* tests returned as positive after cytokine testing had been arranged and set up. 2A computer error during the analysis led to loss of one sample result. Uninfected = all 3 testing methods used, and all negative. Past infection = positive *S. stercoralis* serology with no positive stool testing (but at least one of stool microscopy or stool PCR performed). Active infection = Positive stool microscopy or stool PCR for *S. stercoralis*, regardless of other testing performed. Other=not meeting criteria for the uninfected, past infection, or active infection, groups. N=number of participants undergoing testing

The numbers of each cytokine detected in CSF, by testing plate, are shown in table 3-6.

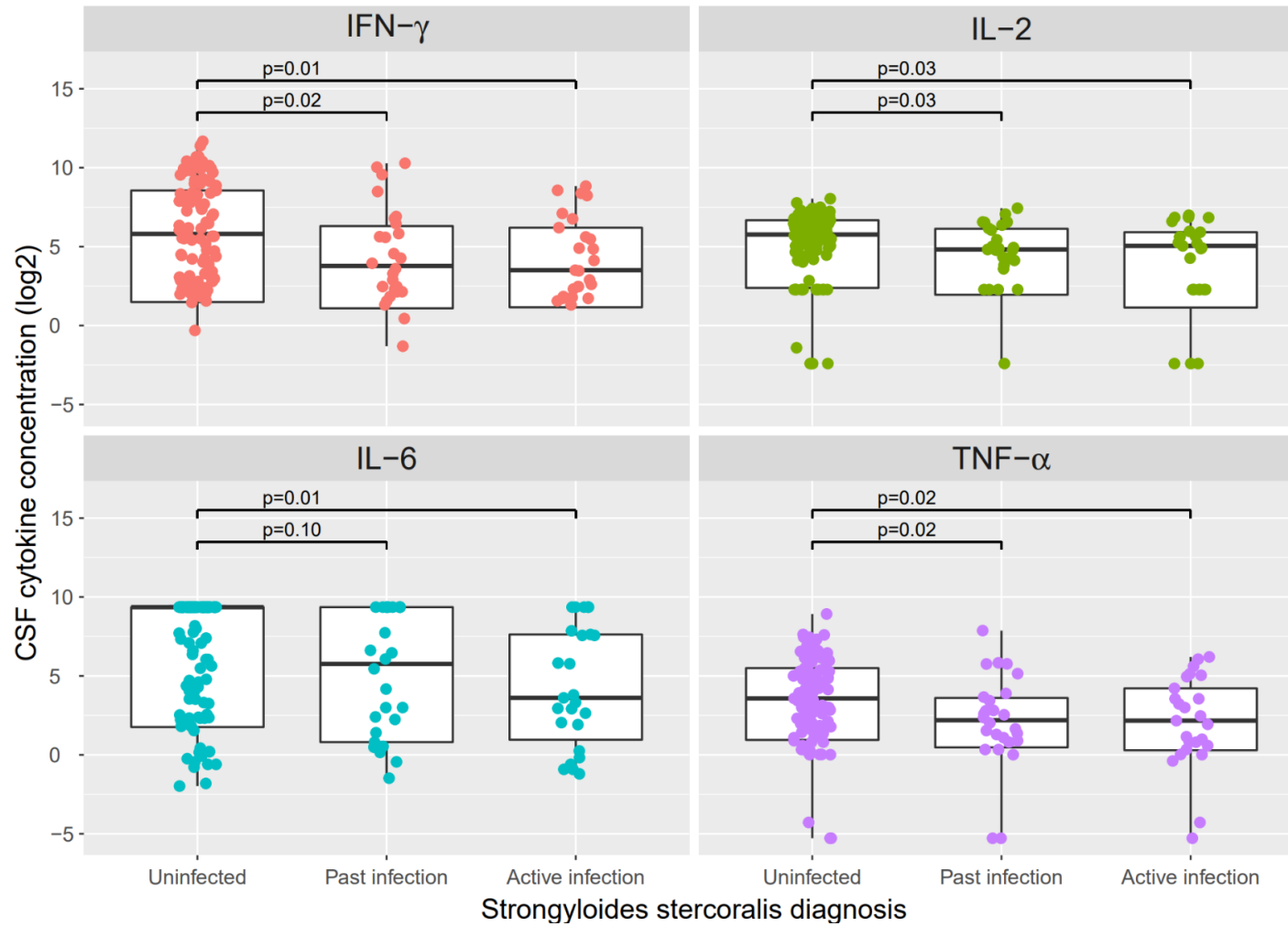
Table 3-6: CSF cytokine detection shown by plate and in total, for 10 tested cytokines

Cytokine	Plate 1 (N=87)		Plate 2 (N=76)		Total (N=163)	
	Detected	Undetected	Detected	Undetected	Detected	Undetected
TNF- α	87	0	71	5	158	5
IFN- γ	87	0	75	1	162	1
IL-1 β	43	44	32	44	75	88
IL-2	64	23	66	10	130	33
IL-4	64	23	76	0	140	23
IL-5	24	63	25	51	49	114
IL-6	87	0	76	0	163	0
IL-10	79	8	68	8	147	16
IL-12p70	32	55	15	61	47	116
IL-13	87	0	39	37	126	37

For IL-6 there were 71 samples with results reported as > 1180 pg/mL. IL-6 was the only cytokine to return values greater than an upper threshold. CSF=cerebrospinal fluid. IL=interleukin. N represents the number of CSF samples tested on each plate.

Log2 CSF concentrations of IFN- γ , IL-2, and TNF- α are shown in figure 3-4.

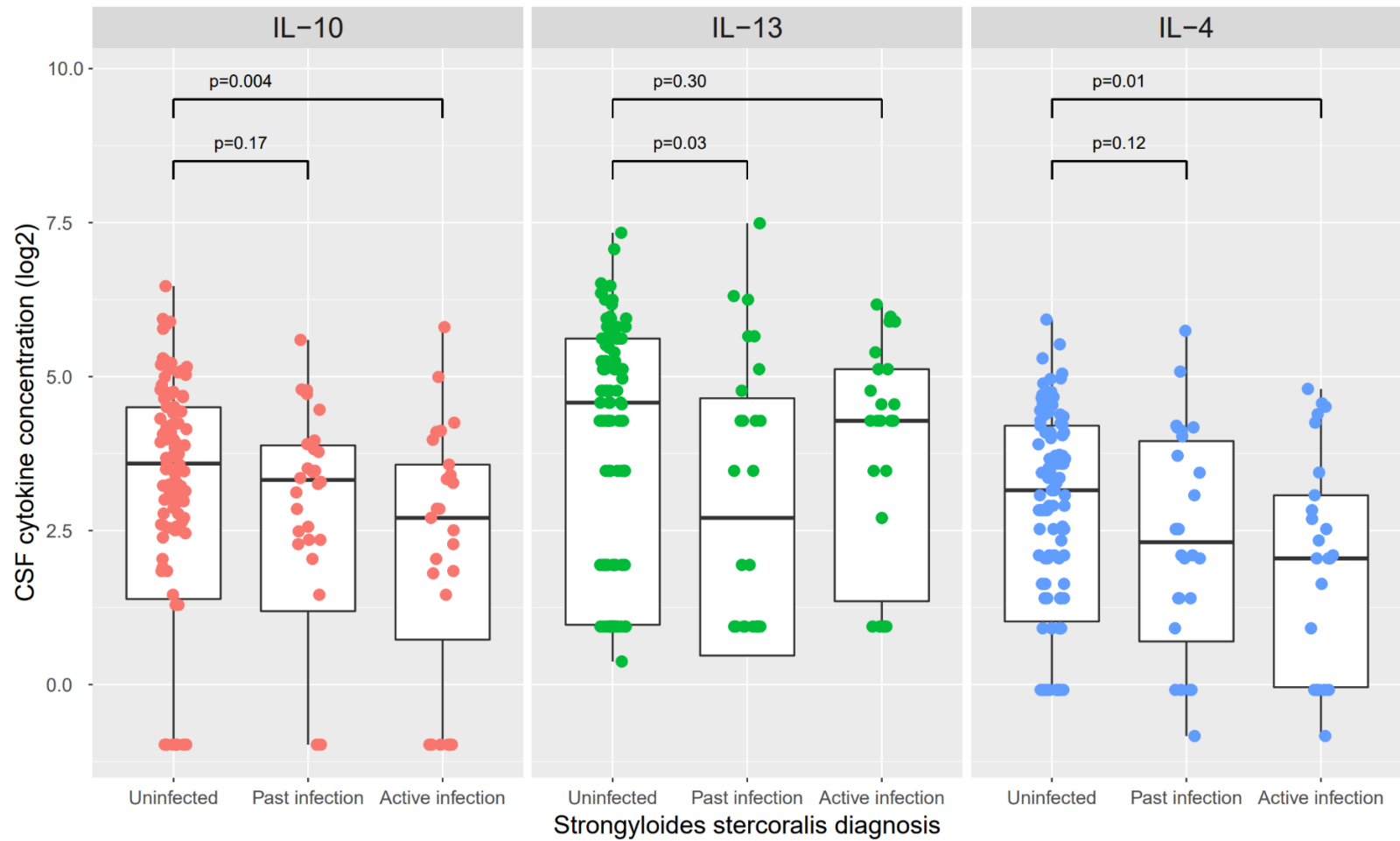
Figure 3-4: Log2 CSF IFN- γ , IL-2 and TNF- α concentrations in participants uninfected with *S. stercoralis*, with past *S. stercoralis* infection, or with active *S. stercoralis* infection



For each individual boxplot, the central horizontal bar represents the median value. The box contains data between 3rd quartile (upper end of box) and 1st quartile (lower end of box). Vertical lines above and below each box extend to the most extreme data point that is within 1.5x the vertical height of the box. Dots represent individual data points. Uninfected = all 3 testing methods used, and all negative. Past infection = positive *S. stercoralis* serology with no positive stool testing (but at least one of stool microscopy or stool PCR performed). Active infection = Positive stool microscopy or stool PCR for *S. stercoralis*, regardless of other testing performed. Cytokine concentrations are shown in pg/mL. Statistical comparison of cytokine concentrations was performed by the Wilcoxon rank sum test. CSF=cerebrospinal fluid

Log2 CSF concentrations of pro-inflammatory cytokines were significantly reduced in participants with active *S. stercoralis* infection (n=25), vs. in uninfected participants (n=105); IFN- γ : 3.51 vs. 5.81, p=0.01; IL-2: 5.05 vs. 5.77, p=0.03; TNF- α : 2.17 vs. 3.58, p=0.02; IL-6: 3.61 vs. 9.36, p=0.01. Additionally log2 CSF concentrations of IFN- γ , TNF- α , IL-2, but not IL-6, were significantly reduced in participants with past *S. stercoralis* infection (n=17) vs. in uninfected participants; IFN- γ : 3.77 vs. 5.81, p=0.02; IL-2: 4.82 vs. 5.77, p=0.03; TNF- α : 2.19 vs. 3.58, p=0.02; IL-6: 5.76 vs. 9.36, p=0.10. Log2 CSF concentrations of IL-10, and IL-13, and IL-4 are shown in figure 3-5.

Figure 3-5: Log2 CSF IL-10, IL-13, and IL-4 concentrations in participants uninfected with *S. stercoralis*, with past *S. stercoralis* infection, or with active *S. stercoralis* infection

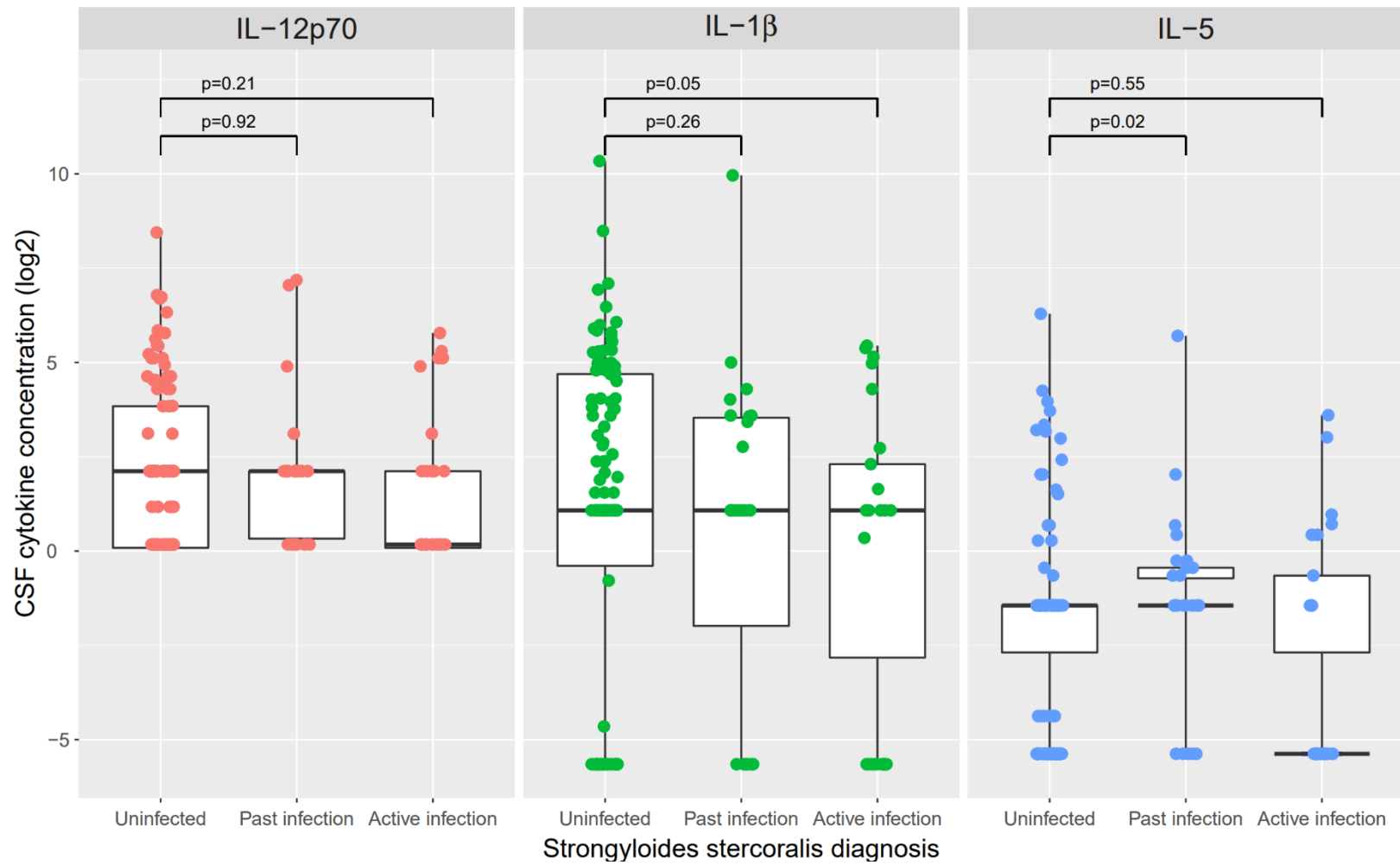


For each individual boxplot, the central horizontal bar represents the median value. The box contains data between 3rd quartile (upper end of box) and 1st quartile (lower end of box). Vertical lines above and below each box extend to the most extreme data point that is within 1.5x the vertical height of the box. Dots represent individual data points. Uninfected = all 3 testing methods used, and all negative. Past infection = positive *S. stercoralis* serology with no positive stool testing (but at least one of stool microscopy or stool PCR performed). Active infection = Positive stool microscopy or stool PCR for *S. stercoralis*, regardless of other testing performed. Cytokine concentrations are shown in pg/mL. Statistical comparison of cytokine concentrations was performed by the Wilcoxon rank sum test. CSF=cerebrospinal fluid

Contrary to our hypothesis, log2 CSF concentrations of IL-10 and IL-4 were significantly reduced in active *S. stercoralis* infection vs. in *S. stercoralis* uninfected participants; IL-10: 2.71 vs. 3.59, $p=0.004$; IL-4: 2.05 vs. 3.15, $p=0.01$. In participants with past *S. stercoralis* infection log2 CSF concentrations of IL-13 were reduced (2.71 vs. 4.58, $p=0.03$), and log2 CSF concentrations of IL-5 were increased (-1.44 vs. -1.44, $p=0.02$), vs. in uninfected participants.

Log2 CSF concentrations of IL-12p70, IL-1 β , and IL-5, for *S. stercoralis* uninfected, past infection and active infection groups, are shown in figure 3-6. Seventy percent of participants had undetectable IL-5 in their CSF samples, hence heavily skewed median values based on interpretation of these undetected values. CSF IL-5 concentrations were significantly higher participants with past *S. stercoralis* infection, vs. *S. stercoralis* uninfected participants, despite identical median values in these groups. Ratio of CSF cytokine change, and statistical comparison between groups, are shown in table 3-7. The ratios of change of each of TNF- α , IFN- γ , and IL-2, were of similar magnitude when comparing uninfected participants to those with past infection, and uninfected participants to those with active infection.

Figure 3-6: Log2 CSF IL-12p70, IL-1 β , and IL-5 concentrations in participants uninfected with *S. stercoralis*, with past *S. stercoralis* infection, or with active *S. stercoralis* infection



For each individual boxplot, the central horizontal bar represents the median value. The box contains data between 3rd quartile (upper end of box) and 1st quartile (lower end of box). Vertical lines above and below each box extend to the most extreme data point that is within 1.5x the vertical height of the box. Dots represent individual data points. Uninfected = all 3 testing methods used, and all negative. Past infection = positive *S. stercoralis* serology with no positive stool testing (but at least one of stool microscopy or stool PCR performed). Active infection = Positive stool microscopy or stool PCR for *S. stercoralis*, regardless of other testing performed. Cytokine concentrations are shown in pg/mL. Statistical comparison of cytokine concentrations was performed by the Wilcoxon rank sum test. CSF=cerebrospinal fluid

Table 3-7: Median log2 CSF cytokine concentrations by testing group

Log2 Cytokine	<i>S. stercoralis</i> testing			Ratio of reduction	
	Uninfected	Past infection	Active infection	Uninfected/ Past infection (Ratio [Wilcoxon])	Uninfected/ Active infection (Ratio [Wilcoxon])
TNF- α	3.58	2.19	2.17	1.63 (p=0.02)	1.65 (p=0.02)
IFN- γ	5.81	3.77	3.51	1.54 (p=0.02)	1.66 (p=0.01)
IL-1 β	1.08	1.08	1.08	1.00 (p=0.26)	1.00 (p=0.05)
IL-2	5.77	4.82	5.05	1.20 (p=0.03)	1.14 (p=0.03)
IL-4	3.15	2.31	2.05	1.36 (p=0.12)	1.54 (p=0.01)
IL-5	-1.44	-1.44	-5.38	1.00 (p=0.02)	0.27 (p=0.55)
IL-6	9.36	5.76	3.61	1.63 (p=0.10)	2.59 (p=0.01)
IL-10	3.59	3.32	2.71	1.08 (p=0.17)	1.32 (p=0.004)
IL-12p70	2.12	2.12	0.17	1.00 (p=0.92)	12.5 (p=0.21)
IL-13	4.58	2.71	4.28	1.69 (p=0.03)	1.07 (p=0.30)

P values are shown for comparison with negative group in each case. The Wilcoxon rank sum test was used to compare cytokine data. Cytokine concentrations are shown in pg/mL.

IL-12p70 and IL-6 concentrations experienced the greatest ratio of reduction (12.5x and 2.6x, respectively) in active *S. stercoralis* infection compared with uninfected participants.

3.3.5 *S. stercoralis* co-infection and outcome from TBM

A comparison of neurological complications by 3 months, and death by 3 months, between primary analysis populations, is shown in table 3-8.

Table 3-8: A comparison of neurological complications and death by 3 months for cytokine testing groups

	<i>S. stercoralis</i> testing				
	Uninfected	Past infection	P value	Active infection	P value
Patients (No.)	110	30		26	
Neurological complications by 3 months					
- Yes (%)	33 (28.2 %)	5 (13.3%)	0.22	1 (3.8%)	0.01
- No (%)	77 (71.8%)	25 (86.7%)		25 (96.2%)	
Death by 3 months					
- Yes (%)	31 (28.2%)	5 (13.3%)	0.30	4 (15.4%)	0.27
- No (%)	79 (71.8%)	25 (86.7%)		22 (84.6%)	

P values are shown for group comparison with *S. stercoralis* uninfected group in each case. The chi squared test was used to compare categorical data. Uninfected = all 3 testing methods used, and all negative. Past infection = positive *S. stercoralis* serology with no positive stool testing (but at least one of stool microscopy or stool PCR performed). Active infection = positive stool microscopy or stool PCR for *S. stercoralis*, regardless of other testing performed.

Neurological complications by 3 months were significantly reduced in participants with active *S. stercoralis* infection (3.8% [1/26]), vs. uninfected participants (28.2% [33/110]) (p=0.01). Neurological complications are listed in Table 3-9.

Table 3-9: Neurological complications in *S. stercoralis* uninfected, past infection, and active infection groups

	<i>S. stercoralis</i> testing		
	Uninfected (N=110)	Past infection (N=30)	Active infection (N=26)
Neurological events (No. [%])	33 (30.0%)	5 (16.7%)	1 (3.8%)
Fall in GCS ≥ 2 points for ≥ 48 hours (No. [%])	26 (23.6%)	4 (13.3%)	0 (3.8%)
Focal neurological sign (No. [%])	5 (4.5%)	0 (0%)	0 (0%)
Seizure (No. [%])	2 (1.8%)	0 (0%)	0 (0%)
Paraplegia/paraparesis (No. [%])	0 (0%)	1 (3.3%)	0 (0%)

N=number of participants. GCS=Glasgow coma score

A fall in GCS ≥ 2 points for ≥ 48 hours was the most common neurological complication recorded, accounting for 80% (4/5) neurological complications in past *S. stercoralis* infection, and 78.8% (26/33) neurological complications in *S. stercoralis* uninfected participants. In a multivariate logistic regression active *S. stercoralis* infection was significantly and independently associated with reduced neurological events by 3 months ($p=0.01$) (table 3-10). Death by 3 months was not significantly reduced between active *S. stercoralis* infection and uninfected groups; 15.4% (4/26) vs. 28.2% (31/110), respectively, $p=0.27$.

Table 3-10: Multivariate analysis of factors predicting neurological complications by 3 months

Variable	Odds Ratio	95% CI	P value
Age (years)	0.99	0.95-1.02	0.38
MRC TBM Grade 2	9.72	3.06-38.7	<0.001
MRC TBM Grade 3	11.2	3.05-49.1	<0.001
HIV co-infection	2.38	0.84-7.03	0.10
Active <i>S. stercoralis</i> co-infection	0.09	0.00-0.54	0.01

Odds ratios with CIs are shown for the prediction of neurological complications with each variable. Age represents an increase in 1 year. MRC TBM Grade 2 and 3 represents comparisons with MRC TBM Grade 1 in each case. HIV co-infection represents comparison with HIV uninfected participants. Active *S. stercoralis* co-infection represents comparison with *S. stercoralis* uninfected participants. Q=quartile. CI=confidence interval. HIV=human immunodeficiency virus. MRC=Medical Research Council. TBM=tuberculous meningitis.

Additional sub-population comparisons of clinical endpoints in participants with and without positive *S. stercoralis* testing are shown in tables 3-11 and 3-12.

Table 3-11: A comparison of neurological complications and death by 3 months in participants who had *S. stercoralis* serology performed

	Negative <i>S. stercoralis</i> serology	Positive <i>S. stercoralis</i> serology	P value
Patients (No.)	606	53	
Neurological complications by 3 months			
- Yes (%)	143 (23.6%)	8 (15.1%)	0.21
- No (%)	463 (76.4%)	45 (84.9%)	
Death by 3 months			
- Yes (%)	156 (25.7%)	8 (15.1%)	0.12
- No (%)	450 (74.3%)	45 (84.9%)	

P values are shown for group comparison with the chi squared test used to compare data.

Table 3-12: A comparison of neurological complications and death by 3 months in participants who had *S. stercoralis* serology and stool microscopy performed

	<i>S. stercoralis</i> testing				
	Group A	Group B		Group C	
	Negative by serology and stool microscopy	Positive by serology and negative by stool microscopy	P value	Positive by both serology and by stool microscopy	P value
Patients (No.)	475	37		7	
Neurological complications by 3 months					
- Yes (%)	106 (22.3%)	4 (8.1%)	0.15	0	0.34
- No (%)	369 (77.7%)	33 (91.9%)		7	
Death by 3 months					
- Yes (%)	111 (23.4%)	4 (10.8%)	0.12	0	0.31
- No (%)	364 (76.6%)	33 (89.2%)		7	

P values are shown for comparison with negative group in each case. The chi squared test was used to compare data.

In participants who had serology performed (n=659), a reduction in death by 3 months was suggested; (15.1% [8/53] vs. 25.7% [156/606] respectively, p=0.12), however these data did not reach statistical significance. When participants who had both *S. stercoralis* serology and stool microscopy performed were compared, neither neurological events by 3 months, nor death by 3 months, were significantly different between participants with positive *S. stercoralis* serology and positive stool microscopy, positive *S. stercoralis* serology but negative stool microscopy, and participants negative for *S. stercoralis*.

3.4 Discussion

TBM is the most severe form of TB and causes death or disability in up to 50% of cases. *S. stercoralis* is a neglected tropical infection with a large global disease and latent carriage

burden. The ability of helminths to modulate the host immune system is well recognised, however immunomodulation of the severe and dysregulated inflammatory response of TBM has not previously been described. In this study of 668 Vietnamese adults with TBM, active *S. stercoralis* infection was associated with a reduced severity of presenting TBM disease, a CSF inflammatory parameter and cytokine profile suggestive of reduced intracerebral inflammation, and reduced neurological complications by 3 months.

In this study 9.2% individuals tested positive for *S. stercoralis* by at least one of serology, stool microscopy, or stool PCR. The true *S. stercoralis* co-infection rate is likely higher, given not all 668 participants received all 3 tests. In particular, in HIV co-infected individuals, *S. stercoralis* serology (the most frequently performed test in this study) may be falsely negative. This high proportion of *S. stercoralis* co-infection is especially concerning given the widespread use of corticosteroid-containing traditional medicines, and the resulting risk of hyperinfection.

The CSF profile of TBM is characterised by elevated total WCC and protein, and reduced glucose (<50% serum glucose), with elevated pro-inflammatory CSF cytokine concentrations.[25,26,221] A predominantly neutrophilic CSF (contrary to the ‘typical’ lymphocytic predominance of TBM) may indicate more intracerebral inflammation, and a higher risk of developing neuroinflammatory complications. In this study active *S. stercoralis* infection was associated with significant reductions in the absolute CSF neutrophil count and the neutrophil proportion, vs. *S. stercoralis* uninfected participants. Additionally, active *S. stercoralis* infection was associated with non-significant reductions in CSF total WCC, CSF protein, and an increase in CSF/blood glucose ratio, vs. *S. stercoralis* uninfected participants. An association between TBM severity and *S. stercoralis* co-infection was also observed. Grade 3 TBM represents the more severe form of TBM. Active *S. stercoralis* infection was associated with a non-significant reduction in grade 3 TBM, compared with grade 3 TBM in *S. stercoralis* uninfected participants.

An analysis of log2 CSF cytokine concentrations supported a conclusion of reduced intracerebral inflammation in active *S. stercoralis* infection. The pro-inflammatory cytokines IFN- γ , IL-2, IL-6, and TNF- α were all significantly reduced with active *S. stercoralis* infection in a pre-treatment CSF analysis.

Interestingly, *S. stercoralis* co-infection was also associated with changes in classical Th2 CSF cytokine responses. CSF IL-4 and IL-10 concentrations were significantly reduced in participants with active *S. stercoralis* co-infection vs. in participants uninfected with *S.*

stercoralis. CSF IL-5 and IL-13 concentrations, however, were not reduced between these *S. stercoralis* groups.

Interplay between Th1 and Th2 pathways are clearly complex, and division of immune responses and cytokines profiles into Th1 and Th2 categories an oversimplification. Suppression of IL-4 and IL-10 does not fit with the hypothesis of reduced ‘pro-inflammatory’ cytokines in the context of active *S. stercoralis*. Further work is required to understand the mechanisms of *S. stercoralis* immunomodulation. Previous data in TBM have in fact shown IL-10 to be elevated in TBM, decreasing after anti-TB chemotherapy.[25,26] The mechanism of cytokine reduction in *S. stercoralis* co-infected TBM be may mediated through reduced CSF neutrophils; neutrophils highly express IL-4 and IL-10 in *M. tuberculosis* infection.[24]

S. stercoralis co-infection in TBM was associated with a significant reduction in neurological complications by 3 months, consistent with presenting severity and CSF parameter data indicating reduced intracerebral inflammation. Reduced neurological complications could not be explained by age, or HIV co-infection. Active *S. stercoralis* co-infection in TBM appears to modulate pro-inflammatory CSF cytokine concentrations, with this immunomodulation conferring neuroprotection. This is a striking finding, although not unexpected. Helminths are known to downregulate classical Th1 immune responses, and in TBM where often a fine line exists between appropriate and excessive inflammation; resulting improved clinical endpoints are feasible. Therapies in severe TBM often attempt to suppress such an excessive host immune response. Corticosteroids are the most widely used adjunctive anti-inflammatory agent, although their benefit may only be short lived, and may not occur in all patient groups. Infliximab and thalidomide, both able to reduce TNF- α , have been proposed as future immunomodulating therapies, yet trial data in TBM is lacking. Our findings raise the question of whether helminth associated immunomodulation can be used to identify novel therapeutic targets for adjunctive therapies in TBM.

How should these results be translated to patient care? It is unknown how treating and eradicating *S. stercoralis* affects subsequent immune responses to TBM. An immune signature may be left which may lead to persistent immunomodulation, or immunomodulatory effects may quickly subside. Duration of *S. stercoralis* infection, worm burden, and anti-helminthic treatments may all impact upon immunomodulation and need further study. It is interesting to consider why *S. stercoralis* has such an effect on CNS diseases and CSF cytokines, given *S. stercoralis* is not routinely a CNS pathogen. *S. stercoralis* does indeed migrate through the body

to the intestine, but this route should not involve the CNS. Only rarely in immunodeficient states does *S. stercoralis* penetrate the blood brain barrier, or the blood-CSF barrier.[237–239]

There are limitations with this study. Our study population may not demographically reflect a typical Vietnamese population; predominance towards males and younger adults may select for a higher risk group for *S. stercoralis*. This, plus the variable numbers of tests performed in participants, makes it difficult to understand the prevalence of *S. stercoralis* in southern Vietnam. The *S. stercoralis* diagnostic tests used in this study are sub-optimal; the sensitivity of stool microscopy for *S. stercoralis* detection is <30% [240] due to intermittent larval shedding in the stool. Stool PCR is more sensitive (~65%)[195], yet *S. stercoralis* DNA is also shed in the stool non-uniformly. *S. stercoralis* serological tests are affected by reduced sensitivity in advanced immunosuppression[201,202] and by persistence of serological positivity despite successful parasite eradication.[195] In a sub-group of participants undergoing *S. stercoralis* serology testing, serology was less likely to be positive in HIV co-infection, a possible result of false negative *S. stercoralis* serology in this group. IL-6 concentrations in the active *S. stercoralis* group were higher than the reference range in many cases; sample and kit availability did not allow for repeat testing with CSF sample dilution. Longer term impacts on neurological complications or death could not be assessed in this study; study follow up was limited to 3 months. Additionally, the study drug allocation (dexamethasone or placebo) of trial participants is not known. However, this will not influence baseline phenotype, or pre-treatment CSF analyses; all of which represent data or sampling prior to study drug administration. Given the randomised study drug allocation (1:1), dexamethasone and placebo are expected to be evenly distributed within each individual analysis population. Finally, given participants positive for *S. stercoralis* by serology alone are treated on a case-by-case basis, there may be inconsistency of *S. stercoralis* eradication therapy between participants.

The strengths of this study are that it is large and prospective. Patients included in this study were enrolled into one of two randomised double blinded placebo-controlled trials, with careful clinical characterisation of TBM and *S. stercoralis* co-infection, treatment protocols, standard operating procedures, and standardised testing and data collection procedures. Analysis of CSF allows observation of disease and immunomodulation at the site of a disease rather than reliance on blood inflammatory changes to assess intracerebral inflammation.

In conclusion, this study showed that *S. stercoralis* co-infection was associated with reduced disease severity, CSF inflammation, and neurological complications by 3 months. *S. stercoralis* may cause immunomodulation of host immune responses of TBM, conferring neuroprotection.

Further understanding of this immunomodulatory process may aid the development of novel host directed therapies to manage excessive and damaging inflammation of TBM.

3.5 Publications related to this chapter

The methods described within this study are published in the research protocols below:

1. Adjunctive dexamethasone for the treatment of HIV-uninfected adults with tuberculous meningitis stratified by Leukotriene A4 hydrolase genotype (LAST ACT): Study protocol for a randomised double blind placebo controlled non-inferiority trial

Joseph Donovan, Nguyen Hoan Phu, Le Thi Phuong Thao, Nguyen Huu Lan, Nguyen Thi Hoang Mai, Nguyen Thi Mai Trang, Nguyen Thi Thu Hiep, Tran Bao Nhu, Bui Thi Bich Hanh, Vu Thi Phuong Mai, Nguyen Duc Bang, Do Chau Giang, Dang Thi Minh Ha, Jeremy Day, Nguyen TT Thuong, Nguyen Nang Vien, Ronald B. Geskus, Tran Tinh Hien, Evelyn Kestelyn, Marcel Wolbers, Nguyen Van Vinh Chau, Guy E. Thwaites
Wellcome Open Research 2018 Mar 20;3:32

2. Adjunctive dexamethasone for the treatment of HIV-infected adults with tuberculous meningitis (ACT HIV): Study protocol for a randomised controlled trial

Joseph Donovan, Nguyen Hoan Phu, Nguyen Thi Hoang Mai, Le Tien Dung, Darma Imran, Erlina Burhan, Lam Hong Bao Ngoc, Nguyen Duc Bang, Do Chau Giang, Dang Thi Minh Ha, Jeremy Day, Le Thi Phuong Thao, Nguyen TT Thuong, Nguyen Nang Vien, Ronald B. Geskus, Marcel Wolbers, Raph L. Hamers, Reinout van Crevel, Mugi Nursaya, Kartika Maharani, Tran Tinh Hien, Kevin Baird, Nguyen Huu Lan, Evelyn Kestelyn, Nguyen Van Vinh Chau, Guy E. Thwaites
Wellcome Open Research 2018 018 Jun 20;3:31

The data contained within this chapter are under review in the following submission:

3. The influence of *Strongyloides stercoralis* co-infection on the presentation, pathogenesis and outcome of tuberculous meningitis

Joseph Donovan, Trinh Thi Bich Tram, Nguyen Hoan Phu, Nguyen Thi Thu Hiep, Vu Thi Thu Van, Dang Thi Hong Mui, Nguyen Thi Han Ny, Ho Dang Trung Nghia, Nguyen Ho Hong Hanh, Le Van Tan, Nguyen Thuy Thuong Thuong, Guy E. Thwaites
Under review at the Journal of Infectious Diseases on the date of PhD submission

Chapter 4

What is the role of optic nerve sheath ultrasound as a non-invasive tool for intracranial pressure monitoring in adults with tuberculous meningitis?

4.1 Introduction

4.1.1 Tuberculous meningitis and raised intracranial pressure

Death results in up to 50% of those with TBM disease,[3–6] largely due to severe neurological complications which are hard to predict and difficult to manage. Hydrocephalus, tuberculomas and other paradoxical neuroinflammation, IRIS and cerebral infarction all contribute to the devastating morbidity and poor outcomes of TBM. Neurological complications of TBM often converge on the same endpoint of raised ICP, which, after compensatory mechanisms are exhausted, quickly leads to coma and death. The true incidence of raised ICP in TBM is not known.[79]

4.1.2 Homeostatic processes and brain compartments

Homeostatic processes seek to control ICP and maintain it within a normal range in the face of neurological insults. Maintenance of normal ICP occurs largely through adjustments in intracranial volumes within brain compartments. Intracranial volume changes are thought to follow the principles of the Monroe Kellie doctrine, which states that volume within the skull is constant and changes in one compartment (brain, CSF or blood) result in compensatory changes in one or both of the other compartments.[241,242] Hydrocephalus of TBM increases the volume of the CSF compartment, whereas inflammatory masses elevate pressure within the brain compartment. Cerebral blood and/or cerebrospinal fluid may exit the skull to attempt to compensate for increased volume elsewhere within the brain. Cerebral blood flow is maintained by the process of autoregulation.[243]

In the average adult, the average intracranial volume is approximately 1600-1700mls, with CSF and blood making up approximately 100-150mls each, and the remaining volume comprising of brain tissue.[244] Normal ICP in adults is less than 15mmHg, with sustained values above this considered pathological.[243] Normal ICP is lower in children than in adults, with variation by age.[245]

4.1.3 Hydrocephalus and cerebrospinal fluid flow

In hydrocephalus there is blockage of CSF flow from its origin in the choroid plexus (cerebroventricular system) to its absorption in the arachnoid granulations (outside the cerebral ventricles). In non-communicating hydrocephalus CSF becomes trapped within the cerebral ventricular system, in the central portion of the brain. In TBM approximately 20% of hydrocephalus is considered non-communicating, due to fourth ventricular outlet or aqueduct obstruction.[15,139] The remaining ‘communicating’ cases are largely due to distal block of CSF flow by tuberculous exudate within the basal cisterns of the brain, rather than blockage within or between the central cerebral ventricles.

This distinction between non-communicating and communicating forms of hydrocephalus is important, but often difficult to make. Performing a lumbar puncture in non-communicating hydrocephalus may create a greater pressure differential between the ‘trapped’ high pressure intraventricular CSF compartment (where CSF is being continually produced) and the extra-ventricular CSF in the lumbar region (where CSF and its pressure can now be removed), resulting in life threatening cerebellar tonsil coning through the foramen magnum. Even in communicating forms of hydrocephalus, pressure is unlikely to communicate equally to all CSF regions due to the thick exudative nature of CSF in early TBM.

4.1.4 Monitoring intracranial pressure in tuberculous meningitis

Early recognition and management of raised ICP is vital in order to minimise intracerebral damage and maintain cerebral perfusion; late recognition may prove too late to have a meaningful effect on clinical outcome. Table 4-1 lists methods for detecting raised ICP in TBM, with further discussion below.

Table 4-1: Methods for detecting raised intracranial pressure in tuberculous meningitis [79]

Non-invasive	Invasive
Clinical assessment including GCS	Lumbar CSF opening pressure
Fundoscopy	Intraventricular catheters
ONSD ultrasound	Intraparenchymal pressure transducers
Transcranial Doppler ultrasound	Subarachnoid bolts
Brain imaging (CT or MRI)	Epidural transducers

CSF=cerebrospinal fluid. CT=computed tomography. GCS=Glasgow coma score.
MRI=magnetic resonance imaging. ONSD=optic nerve sheath diameter.

4.1.4.1 Invasive monitoring

4.1.4.1.1 Invasive device insertion

The gold standard technique for ICP monitoring in brain injury is invasive intracranial monitoring. These invasive techniques require temporary placement of a probe or catheter within the brain tissue or CSF spaces of the brain, and therefore require specialised neurosurgical input at neurosurgical centres. Potential invasive devices include intraventricular catheters, intraparenchymal pressure transducers, subarachnoid bolts or epidural transducers,[79] however these devices are expensive and not available at many centres.[79,246]

4.1.4.1.2 Lumbar cerebrospinal fluid opening pressure

Lumbar puncture is a minimally invasive procedure used to sample CSF. CSF opening pressure can be measured, and this pressure is often used as a surrogate marker of ICP. At lumbar puncture, a transparent single-use manometer is connected to a needle sheath after placement of the sheath in the lumbar subarachnoid space. CSF flows into the manometer, and the height of the CSF column within the manometer is noted; with larger pressures leading to greater height (normal pressure ~6-20cm in manometer). Alternatively, CSF drops per unit time can be counted as a proxy measurement of lumbar CSF pressure. The subarachnoid space represents one continuous pressure compartment, in which pressure should in theory be evenly distributed throughout the brain and spine; however individual pathologies may interfere with CSF flow, creating separate compartments under differing pressures.

In a study of 12 children (mean age 8.5 years) with lumbar and invasive pressure measurements in the United States, a poor correlation was found between lumbar opening pressure and invasive intracranial bolt pressure, with a suggestion that lumbar puncture measurements overestimated pressure.[247] In a study from Sweden, lumbar CSF opening pressure was shown to correlate with ICP measurement via intraparenchymal catheter tip in 10 adults, however crucially all participants had normal pressure hydrocephalus with a communicating CSF system.[248] Few data currently support a positive correlation between lumbar CSF opening pressure and ICP.

4.1.4.2 Non-invasive monitoring

4.1.4.2.1 Clinical assessment

The effects of raised ICP may be visible through patient symptoms (headache, vomiting) and signs (cranial nerve palsies, reduced GCS, and coma). However, these clinical signs may occur late, and an earlier warning system for raised ICP is warranted.

4.1.4.2.2 Fundoscopy

A fundoscope uses light and magnification to view the fundus of the eye and detect changes in the head of the optic nerve (the optic disc). Papilloedema, where the edges of optic nerve head are blurred (normal margins are sharply delineated), may indicate raised ICP. However, similar blurred edge appearances may appear with inflammation of the optic nerve head without raised ICP (termed ‘papillitis’). The success of detecting papilloedema by fundoscopy is highly dependent on operator clinical skill, plus the prior dilation of the pupil by topical medication, widening the hole through which the fundus is viewed thereby improving the view. Importantly the development of papilloedema can lag behind elevation of ICP;[249] therefore fundoscopy is of limited value as a monitoring tool in the acute setting.

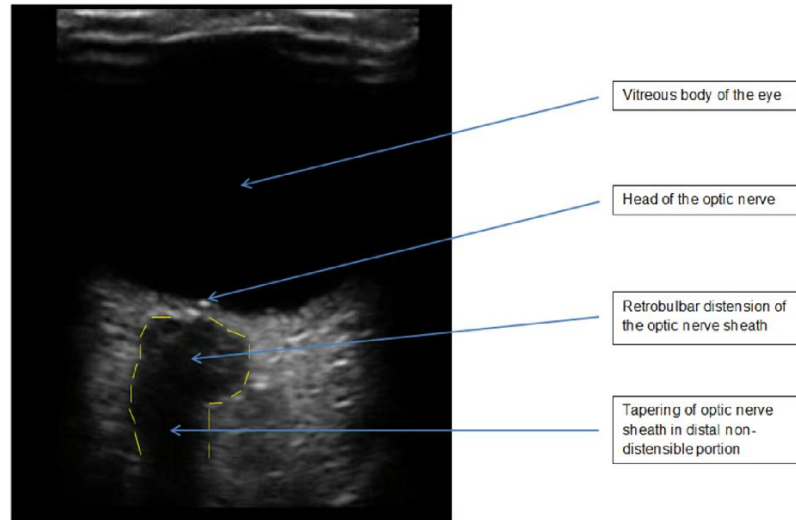
4.1.5 Optic nerve sheath ultrasound

Current non-invasive methods for detecting raised ICP are insufficient. Yet given the particular burden of TB in resource poor settings,[94] affordable, non-invasive and widely available ICP monitoring has the potential to improve TBM patient monitoring globally.

The optic nerve, forming part of the CNS, is surrounded by a dural sheath that is distensible in its retrobulbar segment when ICP is elevated. Whilst papilloedema may develop over hours to days, changes in ONSD due to raised ICP can take only seconds.[250] ‘Real-time’ changes in ONSD have been demonstrated in studies performing ONSD ultrasound 30 minutes after therapeutic lumbar puncture,[251] and only 5 minutes after lumbar puncture.[249] Under ultrasound imaging the optic nerve appears hypoechogenic, closely surrounded by echogenic pia mater, hypoechogenic subarachnoid space, and then hyperechogenic dura mater and periorbital fat.[252] ONSD is measured as the distance inside the dura mater (i.e. the distance from the inside border of the dura mater on one side of the optic nerve, to the inside border of the dura mater on the other side of the optic nerve).[252] Optic nerve sheath imaging, with correlation with surrounding anatomy, is shown in figure 4-1.

Figure 4-1: Distended optic nerve sheath consistent with raised ONSD

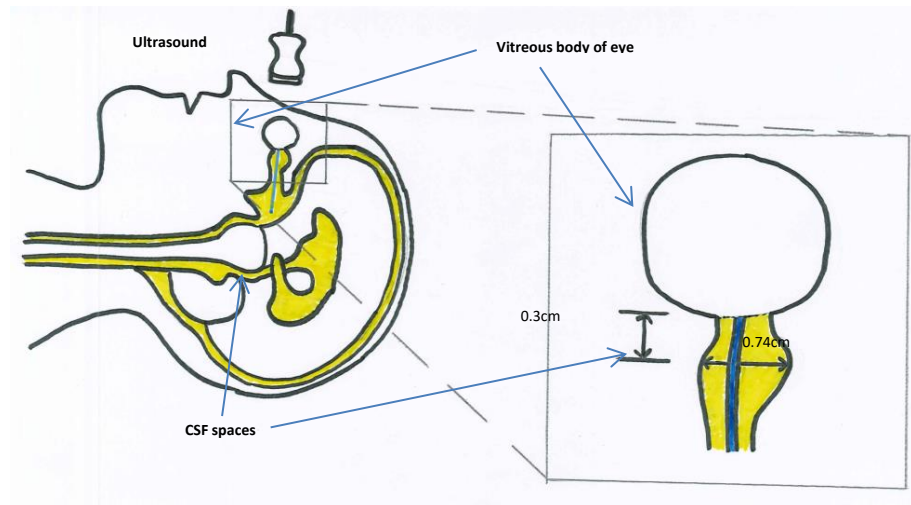
Panel A:



Panel B:



Panel C:



Ultrasound images of the right eye are shown with, (panel A) and without, (panel B) descriptive labels. The borders of the optic nerve sheath are marked with a dotted line in panel A. In panel C a diagram is shown illustrating the appearances seen under ultrasound, showing how these appearances relate to patient position, CSF spaces (yellow) and optic nerve (blue). ONSD measured 0.3cm from the posterior border of the globe of the eye was 0.74cm. CSF=cerebrospinal fluid. ONSD=optic nerve sheath diameter.

4.1.6 Strengths and limitations of ONSD ultrasound

ONSD ultrasound is a safe method of ICP monitoring. Medical ultrasound uses high frequency sound waves to produce an image of an area of the body. Sound waves travel from the ultrasound probe, encountering body tissues which reflect them back to the probe to differing degrees, where they are then converted into an image. Ultrasound scanning modes commonly list mechanical and thermal indices. The mechanical index indicates the relative potential for ultrasound to induce non-thermal adverse events including cavitation, whereas the thermal index indicates the relative potential for tissue temperature rise.[253] British medical ultrasound guidelines advise on safety for ultrasound use in different tissue types.[253] For ultrasound of the eye, a thermal index > 1.0 is not recommended, and a mechanical index > 0.7 may induce cavitation if contrast agents are used (due to destruction of shells of micro-bubbles found in contrast agents).[253] Contrast agents are not used in ONSD ultrasound.

ONSD ultrasound is quick to perform; typically scans can be performed in five minutes. ONSD ultrasound has acceptably low intra-operative and inter-operative variability. A study of 67 healthy individuals who underwent ONSD ultrasound by 3 independent operators showed an average (median) intra-operative variability of $\pm 0.1\text{mm}$, with 5th-95th centile values of $\pm 0-0.4\text{mm}$. After 17 ONSD ultrasound scans were performed the median difference between 2 observers scanning the same patient was no more than 0.3mm (3 comparisons were performed; operator 1 to operator 2, operator 1 to operator 3, operator 2 to operator 3). Median inter-operative variation was $\pm 0.2-0.3\text{mm}$, with 5th-95th centile values of $\pm 0-0.7\text{mm}$.

The first described use of ONSD ultrasound was in the 1980s to differentiate optic nerve lesions.[254,255] B scan ultrasound is now commonly used for ONSD ultrasound. A scan ultrasound may be more accurate for measurements yet this is technically more difficult to perform.[254] Using B scan ultrasound, confounding features may give similar appearances to those associated with raised ICP, such as solid ONS thickening (due to Graves orbitopathy, ONS meningiomas or leukaemic infiltration of the optic nerve) or swelling of the pial and arachnoid sheaths (due to engorged vessels in severe orbital congestion).[255] These conditions are rare however.[256] Ultrasound artefacts may distort or change the image that appears, for example artefact from the multi-layered collagen fibers (lamina cribrosa) through which the optic nerve passes.[255]

4.1.6.1 Standardisation

Little standardisation exists in the field of ONSD ultrasound. No practice guidelines exist, and no clinical training courses specifically focus on this technique. Methods for repeating scans and averaging values differ by study. Elevation of the head of patient bed may affect ICP and should be considered. Where to place measuring calipers has not been defined. Many ultrasound machines have ophthalmic pre-set modes which pre-define mechanical and thermal indices, but not all. In the absence of an ophthalmic mode, ‘best’ indices are uncertain. These uncertainties add to the challenges of performing this technique.

4.1.7 Evidence supporting ONSD ultrasound for ICP monitoring

4.1.7.1 Individual studies

ONSD has been well correlated with invasively measured ICP in individuals with brain injury of a non-infective aetiology. Individual studies of note are discussed further in appendix G. Together these studies show that ONSD correlates well with gold standard ICP monitoring and that an optimal cutoff value for detecting raised ICP is uncertain.

4.1.7.2 Meta-analyses

Three meta-analyses have positively correlated ONSD and invasively measured ICP,[75–77] although studies of Europeans with non-infective pathology have predominated to date. The initial two meta-analyses, performed in 2011 and 2018, included six studies with TBI or intracranial haemorrhage (231 patients)[75] and seven studies (320 patients),[76] respectively. The third and largest systematic review and meta-analysis, performed in 2019, identified an optimal ONSD cut-off of 5mm for the identification of raised ICP in adults and children (71 studies, 4551 patients), however limitations included small study sizes, and no evaluation of clinical outcomes.[77] An individual patient data systematic review is currently underway, in order to identify a definitive cut-off value of ONSD that correlates with an ICP above 20mmHg;[257] results are as yet unpublished. Additionally, a systematic review and meta-analysis specifically comparing ONSD with CT brain-detected raised ICP (rather than invasively measured ICP) showed ONSD to be a high sensitivity tool for ruling out raised ICP in a low-risk group, and highly specific for ruling in raised ICP in a high risk group.[258] Individual studies comparing ONSD with brain imaging suggestive of raised ICP, and the development of brain imaging scoring systems that indicate raised ICP, are described in the appendix (G and H).

4.1.8 Defining a ‘normal’ optic nerve sheath diameter

Defining an optimal cut-off of ONSD to indicate pathology requires an understanding of normal ONSD values. ONSD values in healthy individuals vary by ethnicity.[259] Mean ONSD measurements in healthy patients have been documented as follows; 4.4mm (Turkey),[260] 4.2mm (Korea),[261] 4.4mm (Bangladesh),[262] 4.17-4.1mm (Nigeria),[263] 2.5-4.1mm (United Kingdom),[264] 5.4mm (Germany),[265] 3.6mm (Greece)[266] and 3.68mm (Canada).[267] ONSD does not appear to vary with age, gender, or body mass index,[265,268] with waistline, head circumference, blood pressure or pathological subtype,[268] or side of eye measured.[263] Whilst there appears to be no correlation between ONSD and age in an adult population, a difference in ONSD has been shown between infants and older children (where older age was associated with increasing ONSD).[269] Once a normal range is set for a population, a ROC curve analysis can be performed to set a 'best' cut-off value with sensitivity and specificity for predicting raised ICP.

4.1.9 Evidence supporting ONSD ultrasound for ICP monitoring in brain infection

ONSD ultrasound for ICP monitoring has infrequently been described in individuals with brain infection. In a study of 57 HIV co-infected, ART-naïve patients with suspected meningitis (predominantly cryptococcal) undergoing ONSD ultrasound in Uganda, median ONSD (average across both eyes) was 5.5mm (IQR 5.0-6.0mm) before performing lumbar puncture, and 5.5 mm (IQR 4.9–5.9 mm) after performing lumbar puncture.[270] There was a moderate positive correlation (Spearman correlation coefficient, $\rho=0.44$; $p<0.001$) between ICP (lumbar CSF opening pressure by manometer) and ONSD.[270] Based on ROC curve analysis, an average ONSD taken across both eyes of ≥ 5 mm had a 85% sensitivity and 59% specificity for predicting raised ICP $> 20\text{mmHg}$. [270]

One small study has described ONSD ultrasound in TBM.[78,271] In this study 25 Indian adults with suspected TBM based on consistent brain MRI appearances ($n=25$, mean ONSD 5.81mm) were compared with a control group where individuals lacked MRI appearances of TBM or papilloedema on fundoscopy ($n=120$, upper limit of normal for ONSD 4.37mm).[78] Larger studies are required to further investigate the role of ONSD ultrasound in TBM.

4.1.10 Research questions

ONSD ultrasound has potential value in the detection and monitoring of raised ICP in TBM. Therefore, I set out to establish whether ONSD measured by ultrasound at baseline, correlated with sex, final diagnosis, MRC TBM severity grade, HIV status, plasma sodium, and clinical endpoints by 3 months. Additionally, I sought to correlate ONSD with brain imaging, construct ROC curves

allowing ‘best’ ONSD cut-offs predicting abnormal brain imaging and death, and investigate how ONSD changes during the first 30 days of anti-TB chemotherapy.

4.2 Methods

4.2.1 Study participants

The study was nested within the ACT HIV (clinicaltrials.gov NCT03092817)[85] and LAST ACT (clinicaltrials.gov NCT03100786)[41] clinical trials. Participants in this study were Vietnamese adults (≥ 18 years of age) based only at HTD, Ho Chi Minh City, Vietnam. Inclusion and exclusion criteria, anti-TB chemotherapy, double blinded study drug, ethical approval, and funding, for ACT HIV and LAST ACT, are described earlier in this thesis (chapter 3).

4.2.2 Clinical data

Baseline age, sex, final TBM diagnosis, TBM disease severity (MRC TBM grade) and HIV status were recorded. Participants were followed up for 3 months after enrolment, at which point neurological complications and survival were recorded. Neurological complications were defined as a fall in GCS of ≥ 2 points for ≥ 48 hours, a focal neurological sign, seizure, cerebellar signs, coma, or cerebral herniation.

4.2.3 Brain imaging

Baseline brain MRI or CT imaging was performed in enrolled individuals whenever it was safe and appropriate to do so, and independently reported by an independent UK-based neuroradiologist using a standard template (table 4-2).

Table 4-2: MRI and CT brain reporting template

Study identification number		Enter no. _____
Quality OK?		Yes / No
MRI?		Yes / No
CT?		Yes / No
Normal?		Yes / No
Evidence of bleeding?		Yes / No
Meningeal enhancement?		Yes / No
If yes	Basal	Yes / No
	Sylvian	Yes / No
	Convexity	Yes / No
	Posterior fossa	Yes / No
	Ependymal	Yes / No
Hydrocephalus?		Yes / No
If yes	Communicating	Yes / No
Infarcts?		Yes / No
If yes	Total number	Enter no. _____
	No. diffusion restricted	Enter no. _____
	No. cortical	Enter no. _____
	No. callosal	Enter no. _____
	No. lacunar	Enter no. _____
Tuberculomas?		Yes / No
If yes	Total no.	Enter no. _____
	No. parenchymal	Enter no. _____
	No. ependymal	Enter no. _____
	No. meningeal	Enter no. _____
Local sulcal effacement?		Yes / No

Hemispheric sulcal effacement?		Yes / No
Basal cistern effacement?		Yes / No
Consistent with elevated ICP?		Yes / No
Additional notes?		

CT=computed tomography. ICP=intracranial pressure. ID=identification. MRI=magnetic resonance imaging.

Hydrocephalus, number and location of cerebral infarctions and tuberculomas, meningeal enhancement, and raised ICP features were recorded. For brain MRI, T1, T1 contrast, T1 contrast fluid attenuated inversion recovery (FLAIR) and T2 weighted sequences were performed. Brain imaging was classified as abnormal if it contained one or more of the following: hydrocephalus, cerebral infarction(s), tuberculoma(s), meningeal enhancement and/or features of raised ICP. Regarding allocation of ‘raised ICP’, a yes/no allocation was recorded for each case, based on the global impression of the reporting neuroradiologist, including the presence or absence of sulcal effacement, severity of hydrocephalus, presence of transependymal oedema and any cerebral herniation.

4.2.4 Optic nerve sheath ultrasound

4.2.4.1 Schedule

ONSD ultrasound was performed on days 0, 3, 7, 14, 21 and day 30 (± 1 day) after patient randomisation into ACT HIV or LAST ACT, whenever possible. Day 0 was the day of the taking the first dose of study drug in ACT HIV or LAST ACT, usually also the day of study drug randomisation. Day 30 ultrasound was only performed in participants who remained in hospital at this time point.

4.2.4.2 Timing with brain MRI

ONSD was correlated with brain imaging. MRI facilities were not available at HTD, and participant transport to off-site MRI facilities often took several hours. For participants too unwell for transfer CT brain was performed instead in most cases. A ‘day 0’ ONSD ultrasound was always performed on day 0 ± 1 day. ONSD was correlated with brain imaging that was performed not more than 72 hours before or after that ONSD measurement. This 72 hour time window was practical for critically ill patients, and factored in an off-site MRI scanner, relative visiting times, and maintenance of head of bed elevation during enteral nutrition periods (during which head of bed elevation is often $> 30^\circ$ and unsuitable for this ONSD ultrasound).

4.2.5 Standard procedure

ONSD ultrasound was performed by two clinicians with training in critical care ultrasound, and experience of this scanning technique. Ultrasound was performed using a Sonosite M-Turbo (Fujifilm Sonosite Ltd, Washington, US) or a Lumify (Philips, Amsterdam, Netherlands) ultrasound machine using the following standard procedure:

1. Ensure informed consent obtained for performing ONSD ultrasound in 26TB or 27TB studies. Avoid scanning if visible or known history of ocular trauma.
2. Correctly position patient as able; head-of-bed elevation should be no more than 30° (where 0° is a fully flat bed). Record patient angle.
3. If patient is unconscious, gently turn head to forward facing position. If patient is conscious, ask patient to face forwards, look forwards, and keep eyes closed during scan.
4. Use cleaned L25x 6-13MHz linear probe of M-turbo, or multi-purpose probe of Lumify. Select an appropriate scanning mode (ophthalmic preset). Enter patient identification (ID) in machine; 'ONSD' plus 26TB/27TB study ID plus day ('D') of performing study e.g. ONSD2042D7. Use the 'text' function to apply an 'R' or 'L' to the image screen, thus labelling the image as 'right eye' or 'left eye' respectively. Reduce depth by pressing depth button two times. Do not adjust gain or area of focus.
5. Stand in the most appropriate place for scanning (usually behind the patient head if space available) and position the screen so that it can be seen by the operator.
6. Apply a small volume of ultrasound gel to the ultrasound probe.
7. Place probe gently over temporal portion of the upper eyelid, angling nasally and inferiorly. Ensure pressure is not applied to the eye, and position globe of the eye in the centre of the screen.
8. Identify optic nerve sheath, and then identify where optic nerve is viewed at widest. Scan in both transverse and vertical planes if required. Use 'freeze' button to select and select best view.
9. Perform an assessment of image quality using the following criteria: a) ONS seen to within 1mm of globe of eye, b) 6mm of continuous optic nerve seen, c) Absence of movement artefact. If image quality is considered satisfactory then proceed to qualitative assessment of raised ICP.

10. Perform a qualitative assessment of ICP using the following criteria: a) Retrobulbar bulge of optic nerve seen, b) CSF spaces taper proximally and disappear, c) Papilloedema seen. Assess for the presence or absence of raised ICP, as suggested by a 'yes' score for a, b or c. Proceed to quantitative assessment of ONSD.

11. Measure a 0.3cm distance from most posterior border of the globe of the eye using the caliper measuring function of ultrasound machine. At 3mm from the most posterior part of the globe, measure the diameter of the ONS. This is the distance from echogenic dura mater to echogenic dura mater. Perform 2 measurements for each eye. Record all data on the ONSD case report form. Record image on ultrasound machine and store for later review if required.

4.2.5.1 Recording of ONSD measurements

Previous recording and averaging methods have been described for ONSD (appendix I). For this study ONSD was measured for the right eye twice, and for the left eye twice. An overall average of all four measurements (two right eye and two left eye) was calculated and used for analysis.

4.2.5.2 Non-numerical assessment of ONSD

Each ONSD image was reviewed for quality, before proceeding to the recording of further data. Meeting the following criteria was necessary for measurement: ONS seen to within 1mm of globe of eye, b) 6mm of continuous optic nerve seen, c) absence of movement artefact. In addition, given the unavoidable risk of introducing errors in ONSD measurements, as outlined in the limitations of this scanning technique, additional non-numeric data were collected. Three questions, asked for each eye at each time of scanning, assessed for appearances considered consistent with raised ICP, independent of ONSD size. These questions, chosen based on experience, and expert opinion, were as follows: retrobulbar bulge of optic nerve seen, CSF spaces taper proximally and disappear, papilloedema seen? The presence of any of these three signs was taken to indicate raised ICP.

4.2.6 Pilot data and inter-observer variability

Pilot data was initially collected, with participants and procedures as above, to demonstrate that both ONSD ultrasound operators obtained the same ONSD values from the same patient-eyes, within an acceptable margin of error (i.e. acceptable inter-operative variability). Data from Ballantyne et al,[264] where median average inter-operative variability reduced to 0.3mm with serial scanning, was used to define an acceptable inter-operative variability. A study power calculation[272] was performed for this pilot data; using a significance level of 0.05, a power to

detect the effect size of 0.9, standard deviation (SD) of 0.42 (using TBM ONSD data[78]), and a difference in means of 0.3, a sample size of 23 was calculated. This indicated that if the true difference in the means of matched pairs was 0.3, 23 pairs of ONSD values were required to be able to reject the null hypothesis if it was false. These data showed the inter-operator variability to be acceptable low.

4.2.7 Statistical analysis

4.2.7.1 Allocating test ‘days’

ONSD and sodium values were assigned to days (0, 3, 7, 14, 21, 28) if they were performed on that day, ± 1 day. Day 0 tests are also referred to as ‘baseline’.

4.2.7.2 Sample size

Using a 5% significance level, 90% power to detect effect size, and an expected difference in means of 1.3mm with SD 1.1mm, I calculated 15 patients were required per group (abnormal brain imaging vs. normal brain imaging) to reject the null hypothesis if it were false. This sample size calculation was based on data from a non-TBM study,[273] given the limited TBM data for this purpose. This study,[273] which compared ‘brain pathology with raised ICP confirmed by CT’ (mean 5.4mm, SD 1.1mm) to ‘brain pathology without raised ICP confirmed by CT’ (mean 4.1mm, SD 0.5mm), contained more conservative data (wider SD and smaller difference in means) than many other studies. ONSD changes between brain pathology groups are uncertain within lower or higher TBM severity grades, or between HIV co-infection and HIV uninfected. Therefore, comparisons within these subgroups were exploratory.

4.2.7.3 Statistical analysis plan

A statistical analysis plan written in advance of data analysis outlined the following:

1. Evaluate median baseline ONSD, by sex, final TBM diagnosis, MRC TBM grade, and HIV co-infection status
2. Investigate whether there is a positive correlation between ONSD and brain imaging consistent with raised ICP, or with abnormal brain imaging appearances
3. Establish whether a ROC curve can be constructed to select an ONSD cut-off value predictive of raised ICP in TBM
4. Describe the association between ONSD and plasma sodium measurement, stratified by HIV co-infection, and clinical endpoints

5. Establish whether non-quantitative ONSD appearances (ultrasound operator impression of abnormal optic nerve sheath changes) correlates with brain imaging consistent with raised ICP
6. Correlate ONSD with neurological complications by 3 months, and with death by 3 months
7. Describe ONSD trends during the first 30 days of TBM treatment, stratified by HIV co-infection, TBM severity grade, and clinical endpoints

After data collection, due to the few cases reported as showing ‘raised ICP’ by imaging (classification of imaging as showing raised ICP is challenging as this has limitations), I investigated whether there was a positive correlation between ONSD (or non-quantitative ONSD appearances), with abnormal brain imaging appearances. In addition, I constructed ROC curves to identify ONSD cut-off values predictive of abnormal brain imaging, or of death by 3 months.

Given correlation between baseline ONSD and disease severity, I also analysed the correlation between baseline ONSD and baseline CSF parameters indicating inflammation or severe disease. Disease severity and CSF parameters were also shown for the abnormal brain imaging, and normal brain groups, for whom an association with ONSD had been seen. This allowed me to further explore the relationship between elevated ONSD and neuroinflammation.

4.2.7.4 Statistical tests

Comparison between proportional data was assessed by the chi squared test. Non-normally distributed data were compared using the Wilcoxon rank sum test. Correlation between continuous variables was performed using Spearman’s rank correlation co-efficient. Data were analysed using R (version 3.6).

4.3 Results

4.3.1 Study population

From June 2017 to December 2019 inclusive, 107 Vietnamese adults with TBM had 267 ONSD ultrasound scans performed at day 0 (n=72), day 3 (n=48), day 7 (n=45), day 14 (n=44), day 21 (n=42) and day 30 (n=16). Four images were recorded at each of these 267 scanning time points. Median age of the study population was 37 (IQR 29-45) years. 68.2% (73/107) participants were male and 31.8% (34/107) were female. Final diagnoses of the study population were as follows; 75.7% (81/107) definite TBM, 12.1% (13/107) probable TBM, and 12.1% (13/107) possible TBM. Modified MRC TBM severity grades were; Grade 1: n=33, Grade 2: n=58, Grade 3: n=16. 32.7% (35/107) participants had HIV co-infection. ONSD measurements ranged from 0.38-0.74cm.

4.3.2 Baseline ONSD associations

Baseline ONSD was performed in 67.3% (72/107) participants. Median baseline ONSD is shown by sex, final diagnosis, MRC TBM grade, and HIV co-infection status in table 4-3.

Table 4-3: Median baseline ONSD by sub-categories

	Total No. (N=72)	Median ONSD (cm) (IQR)	P value
All patients	72	0.53 (0.49-0.57)	
Sex			
- Male (No [%])	47 (65.3%)	0.52 (0.49-0.57)	0.89
- Female (No [%])	25 (34.7%)	0.54 (0.48-0.57)	
Final diagnosis (No [%])			
- Definite TBM	53 (73.6%)	0.54 (0.49-0.57)	0.25*
- Probable TBM	7 (9.7%)	0.52 (0.50-0.55)	
- Possible TBM	12 (16.7%)	0.51 (0.49-0.55)	
MRC TBM Grade (No [%])			
- 1	23 (31.9%)	0.50 (0.48-0.54)	0.01 #
- 2	39 (54.2%)	0.55 (0.49-0.57)	
- 3	10 (13.9%)	0.56 (0.52-0.58)	
HIV status (No [%])			
- Positive	20 (27.8%)	0.56 (0.49-0.58)	0.17
- Negative	52 (72.2%)	0.52 (0.49-0.55)	

* Definite TBM compared with non-definite. # Grade 1 compared with grades 2 & 3. The Wilcoxon rank sum test was used to compare ONSD values. Total No. reflects the total number of observations available for the corresponding variable. Medical Research Council grades are as follows: Grade 1 indicates a GCS of 15 with no neurological signs, grade 2 a GCS of 11 to 14 (or 15 with focal neurological signs), and grade 3 a GCS of 10 or less. HIV=human immunodeficiency virus. IQR=interquartile range. MRC= Medical Research Council. ONSD=optic nerve sheath diameter. TBM=tuberculous meningitis.

Baseline ONSD significantly increased with more severe disease (grade 1: 0.50cm, grade 2: 0.55cm, grade 3: 0.56cm), p=0.01. HIV co-infection was not significantly associated with increased ONSD at baseline (table 4-3). No significant correlation was seen between baseline ONSD and

baseline disease temperature, lumbar CSF opening pressure, CSF white blood cells, CSF lactate, CSF protein, or CSF/blood glucose ratio (table 4-4).

Table 4-4: Correlation of baseline ONSD with baseline disease severity and CSF inflammatory parameters

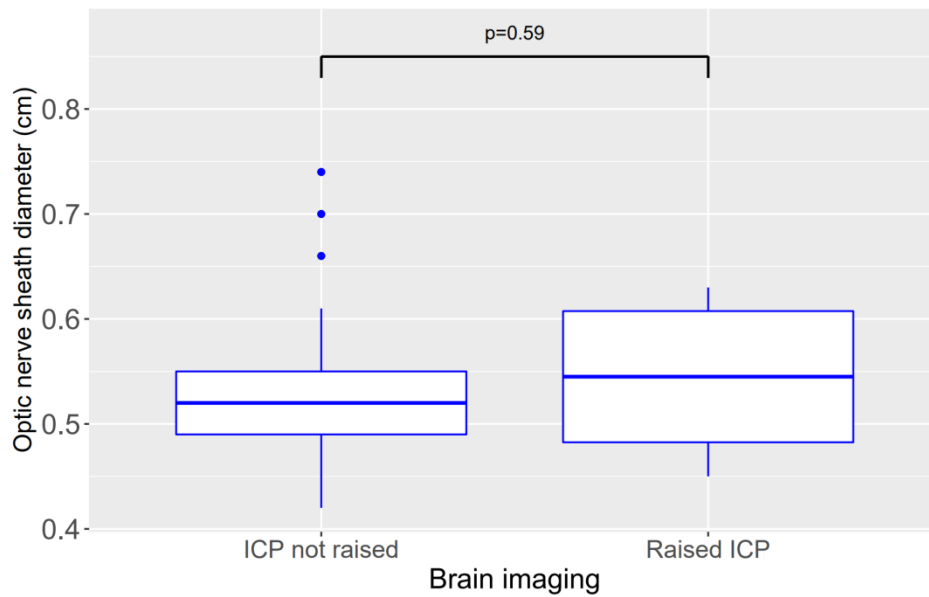
Parameter	Total No.	Correlation co-efficient	P value
Highest temperature (°C)	71	0.08	0.49
Plasma sodium (mmol/L)	65	-0.09	0.48
Lumbar CSF opening pressure (cmH ₂ O)	49	0.09	0.56
CSF WBC (cells/mm ³)	72	0.08	0.52
CSF neutrophils (%)	71	0.23	0.05
CSF neutrophil count (cells/mm ³)	71	0.15	0.22
CSF/blood glucose ratio	71	-0.05	0.65
CSF protein (g/L)	72	0.11	0.38
CSF lactate (mmol/L)	72	0.12	0.31

Co-efficient and significance (p value) are shown using Spearman's rank correlation co-efficient. Highest temperature represents the highest temperature on the day of baseline assessment. CSF=cerebrospinal fluid. ONSD=optic nerve sheath diameter. WBC=white blood cells.

4.3.3 The association between ONSD and brain imaging

I set out to investigate whether increased ONSD correlated with brain imaging consistent with raised ICP, or with abnormal brain imaging appearances. There were 63 participants for whom ONSD and brain imaging were performed within 72 hours of each other at the start of treatment. In 9.5% (6/63) participants brain imaging suggested raised ICP, and in 90.4% (57/63) participants brain imaging did not suggest raised ICP. Median ONSD for the raised ICP and non-raised ICP groups were 0.55cm and 0.52cm respectively, p=0.59 (figure 4-2).

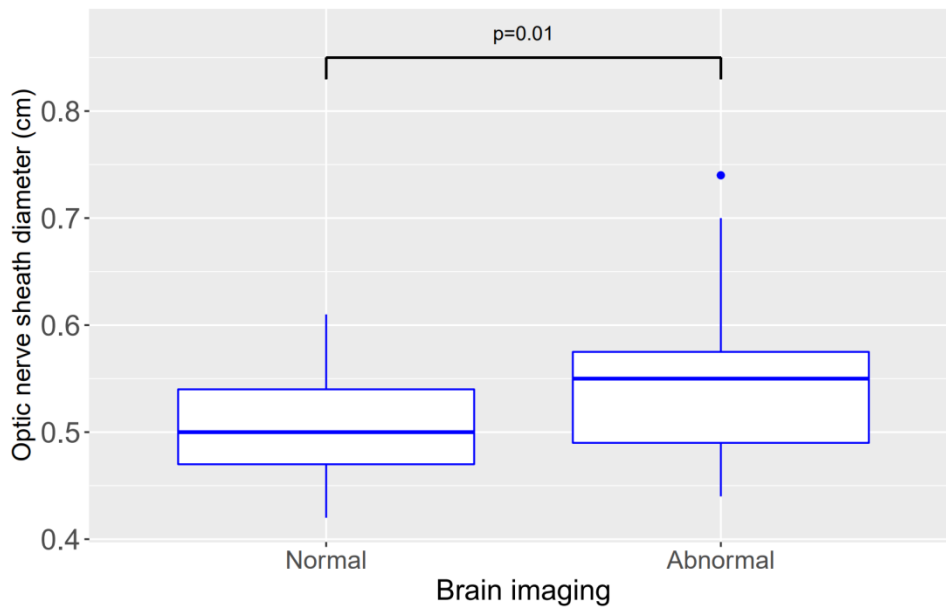
Figure 4-2: ONSD in participants with brain imaging not suggestive of raised ICP vs. in participants with brain imaging suggestive of raised ICP



ICP=intracranial pressure

In this same group of 63 participants, 61.9% (39/63) participants had brain imaging with abnormal appearances consistent with TBM, and 38.1% (24/63) participants had normal brain imaging. Median ONSD for the abnormal imaging and normal imaging groups were 0.55cm and 0.50cm respectively ($p=0.01$) (figure 4-3).

Figure 4-3: Box plot of ONSD values in those with normal brain imaging vs. in those with abnormal brain imaging



ONSD=optic nerve sheath diameter

Median ONSD values by brain pathology groups were as follows; hydrocephalus: 0.55cm (n=10), tuberculoma(s): 0.52cm (n=13), cerebral infarction(s): 0.55cm (n=18), meningeal enhancement: 0.52cm (n=29). To further investigate the difference in ONSD between normal and abnormal brain imaging groups, TBM severity and CSF inflammatory parameters were compared between these two groups (table 4-5).

Table 4-5: Disease severity and CSF inflammatory parameters for normal and abnormal brain imaging groups

	Total No.	Normal brain imaging (N=24)	Total No.	Abnormal brain imaging (N=39)	P value
Age (Median[IQR])	24	41 (28-50)	39	34 (29-40)	0.08
Sex	24		39		1.0
- Male (No [%])		16 (66.7%)		25 (64.1%)	
- Female (No [%])		8 (33.3%)		14 (35.9%)	
MRC TBM Grade (No [%])	24		39		0.09*
- 1		12 (50%)		10 (25.6%)	
- 2		11 (45.8%)		25 (64.1%)	
- 3		1 (4.2%)		4 (10.3%)	
HIV status (No [%])	24		39		1.0
- Positive		6 (25%)		9 (23.1%)	
- Negative		18 (75%)		30 (76.9%)	
Highest temperature (°C) (Median[IQR])	24	38.7 (38.0-39.5)	39	39.0 (38.7-39.5)	0.31
Lumbar CSF opening pressure (cmH ₂ O) (Median[IQR])	15	18 (15-24)	26	20 (17-29)	0.54
CSF WBC (cells/mm ³) (Median[IQR])	24	283 (137-501)	39	304 (200-538)	0.32
CSF neutrophil % (Median[IQR])	23	13 (11-30)	39	48 (20-73)	0.002
CSF neutrophil count (cells/mm ³)	23	43	39	142	0.01

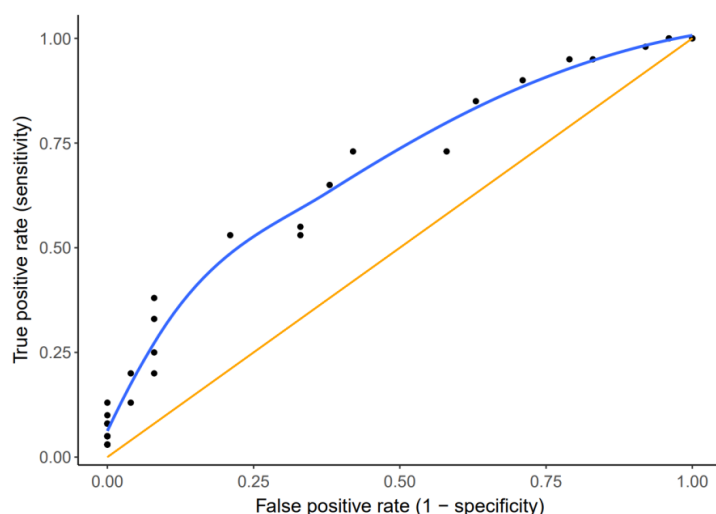
(Median[IQR])		(19-120)		(39-229)	
CSF/blood glucose ratio (Median[IQR])	24	0.39 (0.24-0.44)	39	0.27 (0.23-0.36)	0.02
CSF lactate (mmol/L) (Median[IQR])	24	4.0 (3.0-5.7)	39	5.8 (4.7-7.7)	0.001

* Grade 1 compared with grades 2 & 3. The Wilcoxon rank sum test and chi squared test were used to compare averages of continuous and categorical data, respectively. Highest temperature, lumbar CSF opening pressure, CSF WBC, CSF neutrophil percentage, CSF/blood glucose ratio and CSF lactate are non-normally distributed and are shown as median (IQR). Highest temperature represents the highest temperature on the day of baseline assessment. CSF=cerebrospinal fluid. HIV=human immunodeficiency virus. IQR=interquartile range. MRC=Medical Research Council. TBM=tuberculous meningitis. WBC=white blood cells.

In the abnormal brain imaging group, there were significantly elevated neutrophil percentage, absolute CSF neutrophils, and CSF lactate, and significantly reduced CSF/blood glucose ratio, consistent with increased disease severity in this group.

I then set out to establish whether a ROC curve could be constructed to select an ONSD cut-off value predictive of abnormal brain imaging in TBM, with clinically acceptable sensitivity and specificity values. A ROC table was compiled using 63 pairs of ONSD and brain imaging data (figure 4-4). Clinically acceptable values (i.e. sufficiently sensitive and specific for clinical utility) could not be found for the prediction of abnormal brain imaging.

Figure 4-4: ROC curves for the prediction of abnormal brain imaging in TBM



ROC=receiver operating characteristic. TBM=tuberculous meningitis

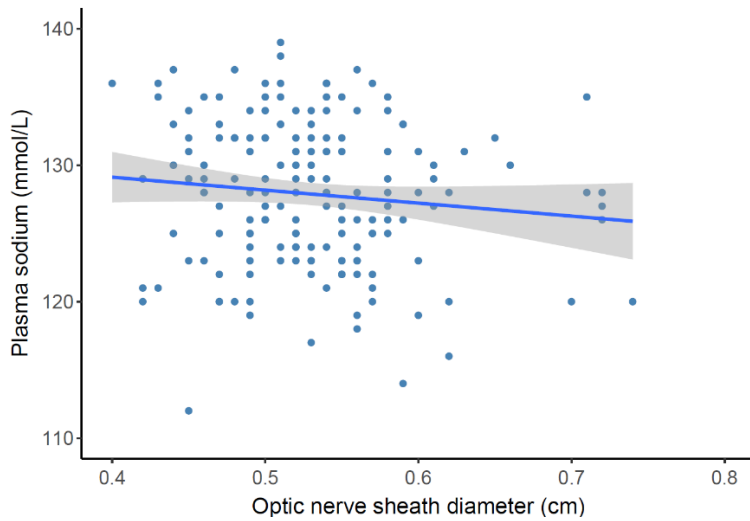
4.3.4 The association of non-quantitative optic nerve sheath diameter with brain imaging

Optic nerve sheath appearances suggestive of elevated ICP (independent of ONSD) were compared with brain imaging. The presence of any of the following features indicated raised ICP; a retrobulbar bulge of optic nerve seen, CSF spaces tapering proximally and disappearing, papilloedema seen. An ultrasound operator impression of raised ICP (as above) vs. no raised ICP, did not correlate with abnormal brain imaging (vs. normal brain imaging) ($p=0.96$).

4.3.5 The association of optic nerve sheath diameter with plasma sodium

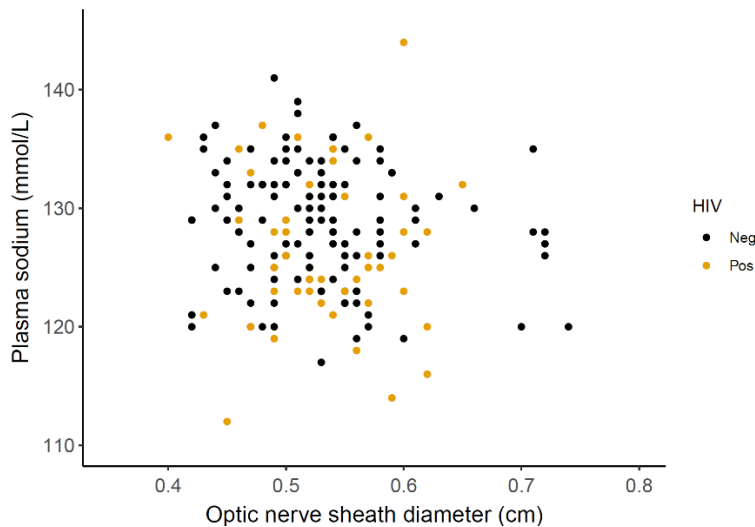
155 paired ONSD and plasma sodium measurements were analysed, taken from 82 individual participants. No significant correlation was seen between ONSD and paired sodium measurements (correlation co-efficient -0.13 [95% CI -0.28-0.03], $p=0.10$). Paired data points (performed on the same 'day') are shown below in figure 4-5, figure 4-6 (colour stratified by HIV co-infection), and figures 4-7 and 4-8 (stratified by clinical endpoints).

Figure 4-5: The association of optic nerve sheath diameter and plasma sodium in TBM



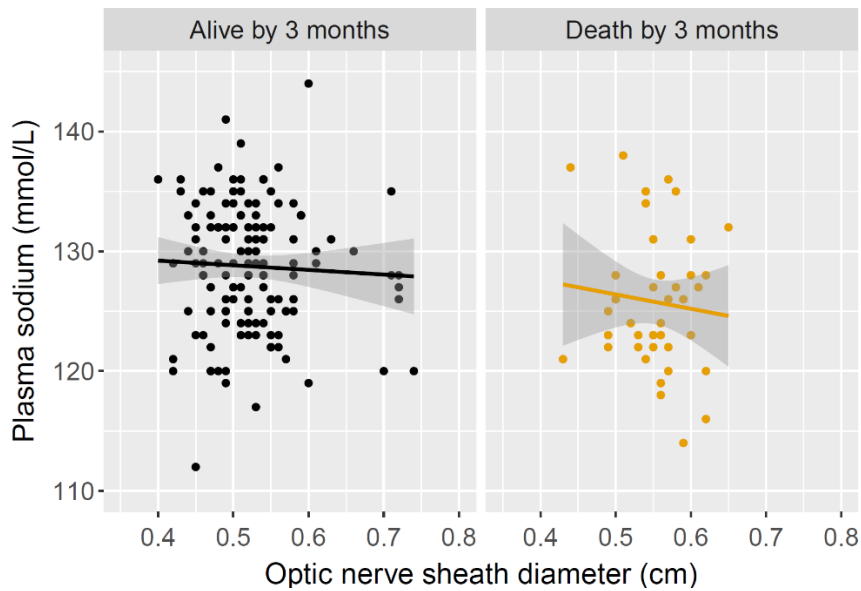
Each dot represents a pair of ONSD and plasma sodium measurements (performed on the same ‘day’). Line of best fit shown with surrounding 95% confidence interval in shading. ONSD=optic nerve sheath diameter. TBM=tuberculous meningitis.

Figure 4-6: The association of optic nerve sheath diameter and plasma sodium in TBM, stratified by HIV co-infection status



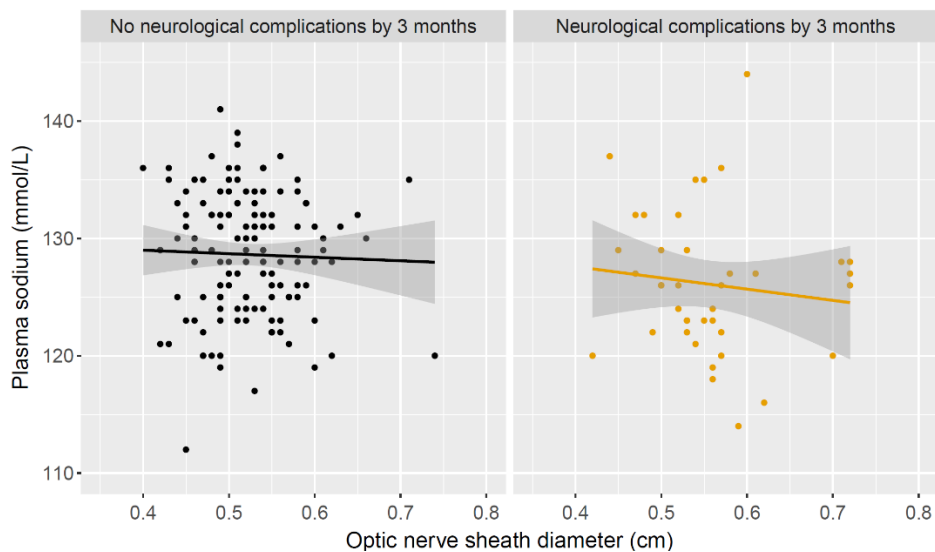
Each dot represents a pair of ONSD and plasma sodium measurements (performed on the same ‘day’). HIV=Human immunodeficiency virus. Neg=negative. ONSD=optic nerve sheath diameter. Pos=positive. TBM=tuberculous meningitis

Figure 4-7: The association of optic nerve sheath diameter and plasma sodium in TBM, stratified by death by 3 months



Each dot represents a pair of ONSD and plasma sodium measurements (performed on the same 'day'). Line of best fit shown with surrounding 95% confidence interval in shading. ONSD=optic nerve sheath diameter. TBM=tuberculous meningitis.

Figure 4-8: The association of optic nerve sheath diameter and plasma sodium in TBM, stratified by neurological complications by 3 months



Each dot represents a pair of ONSD and plasma sodium measurements (performed on the same 'day'). Line of best fit shown with surrounding 95% confidence interval in shading. ONSD=optic nerve sheath diameter. TBM=tuberculous meningitis.

4.3.6 Response to treatment, and outcomes by 3 months

Baseline ONSD was significantly higher in participants who died (0.56cm [15/72]) vs. in participants who survived (0.52cm [57/72]), $p=0.02$ (table 4-6).

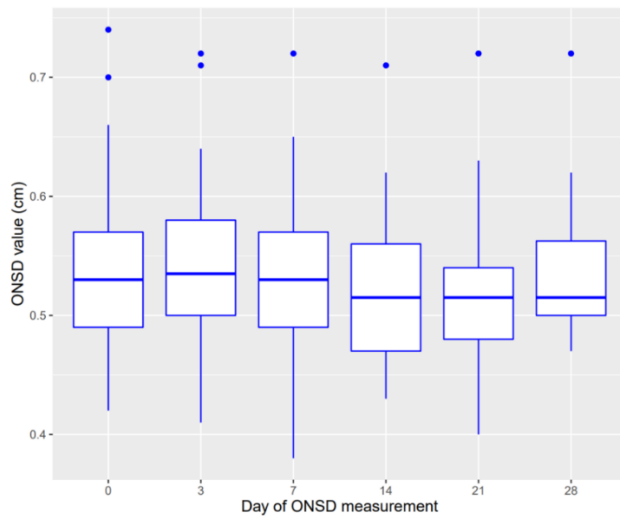
Table 4-6: Median baseline ONSD by clinical endpoints

	Total No. (N=72)	Median ONSD (cm) (IQR)	P value
Neurological complication by 3 months (No. [%])			
- Yes	12 (16.7%)	0.53 (0.51-0.57)	0.61
- No	60 (83.3%)	0.53 (0.49-0.56)	
Death by 3 months (No. [%])			
- Yes	15 (20.8%)	0.56 (0.53-0.59)	0.02
- No	57 (79.2%)	0.52 (0.48-0.56)	

P values represent comparison for ONSD values by the Wilcoxon rank sum test. Total No reflects the total number of observations available for the corresponding variable. Medical Research Council grades are as follows: Grade 1 indicates a GCS of 15 with no neurological signs, grade 2 a GCS of 11 to 14 (or 15 with focal neurological signs), and grade 3 a GCS of 10 or less. ONSD=optic nerve sheath diameter.

At least one ONSD value was recorded for 107 individual participants (figure 4-9).

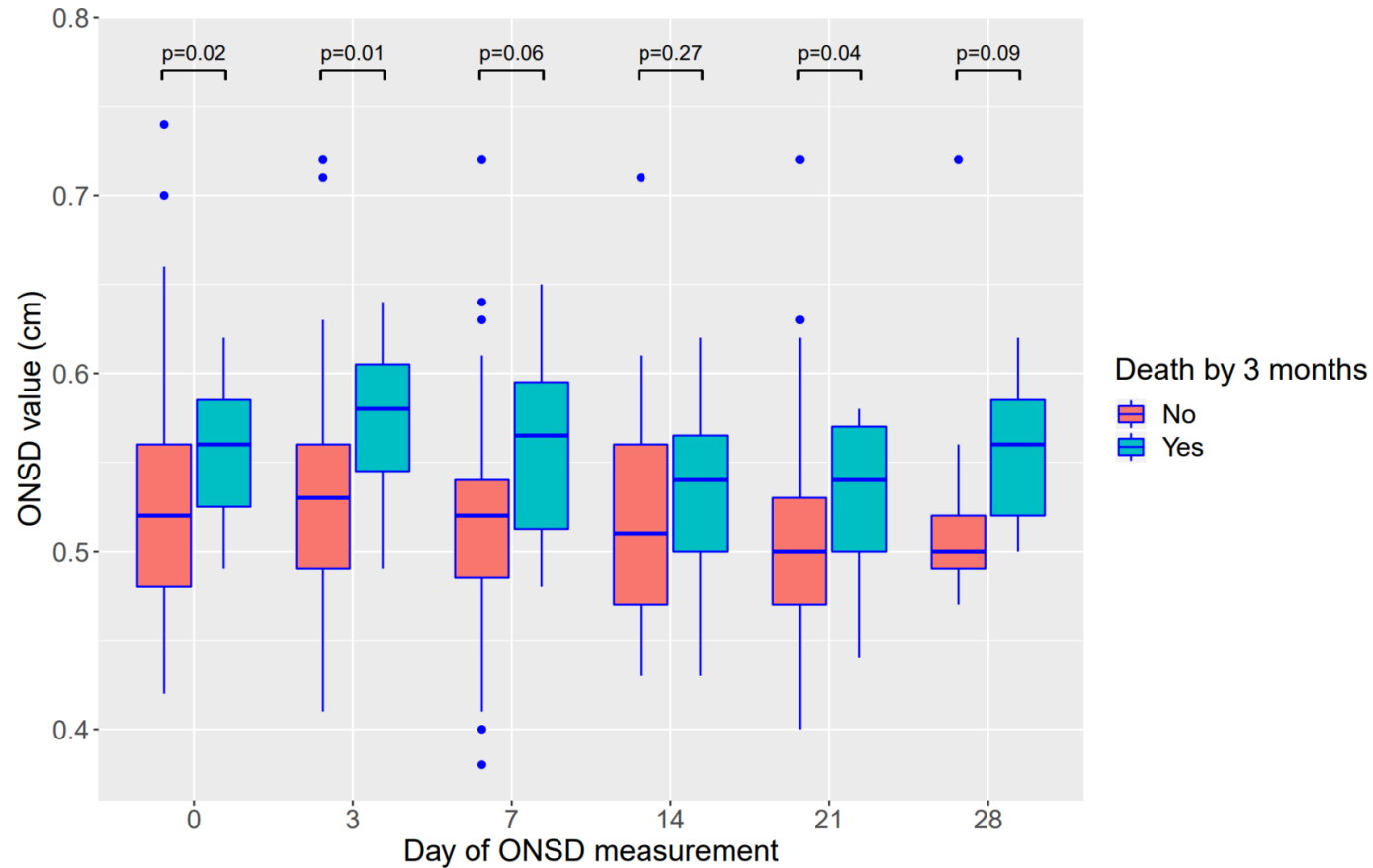
Figure 4-9: Optic nerve sheath diameter by day of measurement



For each individual boxplot, the central horizontal bar represents the median value. The box contains data between 3rd quartile (upper end of box) and 1st quartile (lower end of box). Vertical lines above and below each box extend to the most extreme data point that is within 1.5x the vertical height of the box. Dots represent individual data points outside of these limits. ONSD=optic nerve sheath diameter.

Median ONSD was higher in participants who died by 3 months, vs. in participants who survived by 3 months, at all follow up time points (days 3, 7, 14, 21 and 28) (figure 4-10), and significantly so at baseline, day 3 and day 21 (figure 4-11).

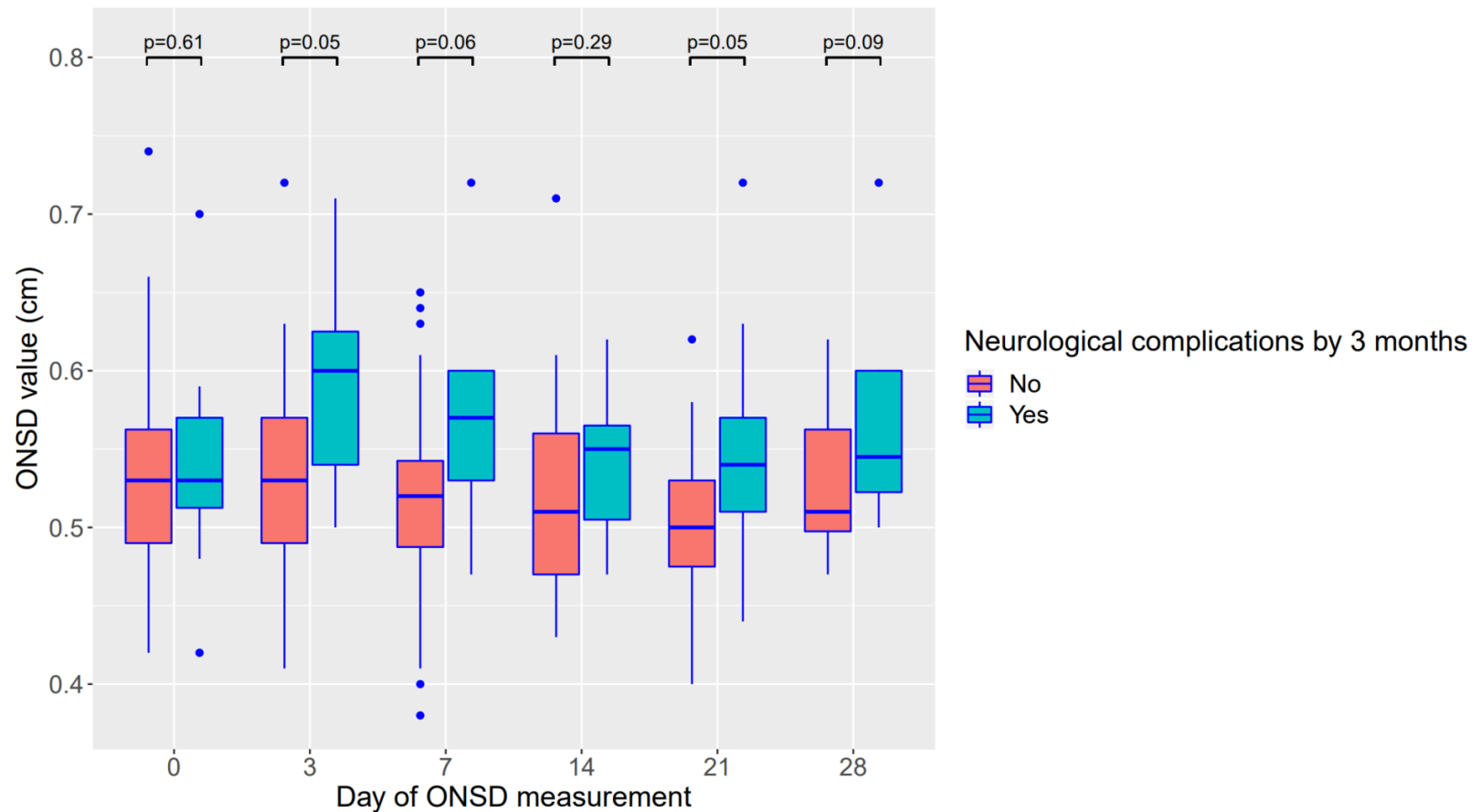
Figure 4-10: ONSD over 30 days of anti-TB chemotherapy, stratified by death by 3 months



For each individual boxplot, the central horizontal bar represents the median value. The box contains data between 3rd quartile (upper end of box) and 1st quartile (lower end of box). Vertical lines above and below each box extend to the most extreme data point that is within 1.5x the vertical height of the box. Dots represent individual data points outside of these limits. P values represent statistical comparison of ONSD values performed by the Wilcoxon rank sum test. ONSD=optic nerve sheath diameter. TB=tuberculosis.

There was no significant difference in baseline ONSD between participants who experienced neurological complications by 3 months, vs. participants who did not (0.53cm vs. 0.53cm, respectively, $p=0.61$). Follow up data suggested a trend of higher ONSD in participants experiencing neurological events by 3 months vs. participants without neurological events (figure 4-11).

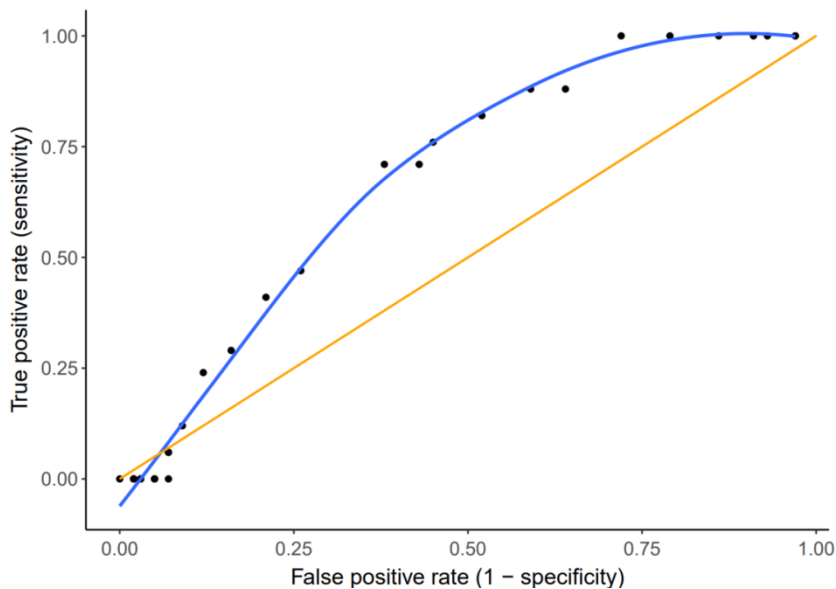
Figure 4-11: ONSD over 30 days of anti-TB chemotherapy, stratified by neurological complications by 3 months



For each individual boxplot, the central horizontal bar represents the median value. The box contains data between 3rd quartile (upper end of box) and 1st quartile (lower end of box). Vertical lines above and below each box extend to the most extreme data point that is within 1.5x the vertical height of the box. Dots represent individual data points outside of these limits. P values represent statistical comparison of ONSD values performed by the Wilcoxon rank sum test. ONSD=optic nerve sheath diameter. TB=tuberculosis.

A ROC curve was constructed to investigate whether ONSD could predict death by 3 months (figure 4-12) with acceptable sensitivity and specificity. A baseline ONSD of 0.53cm or above predicted death by 3 months with 73% sensitivity and 54% specificity.

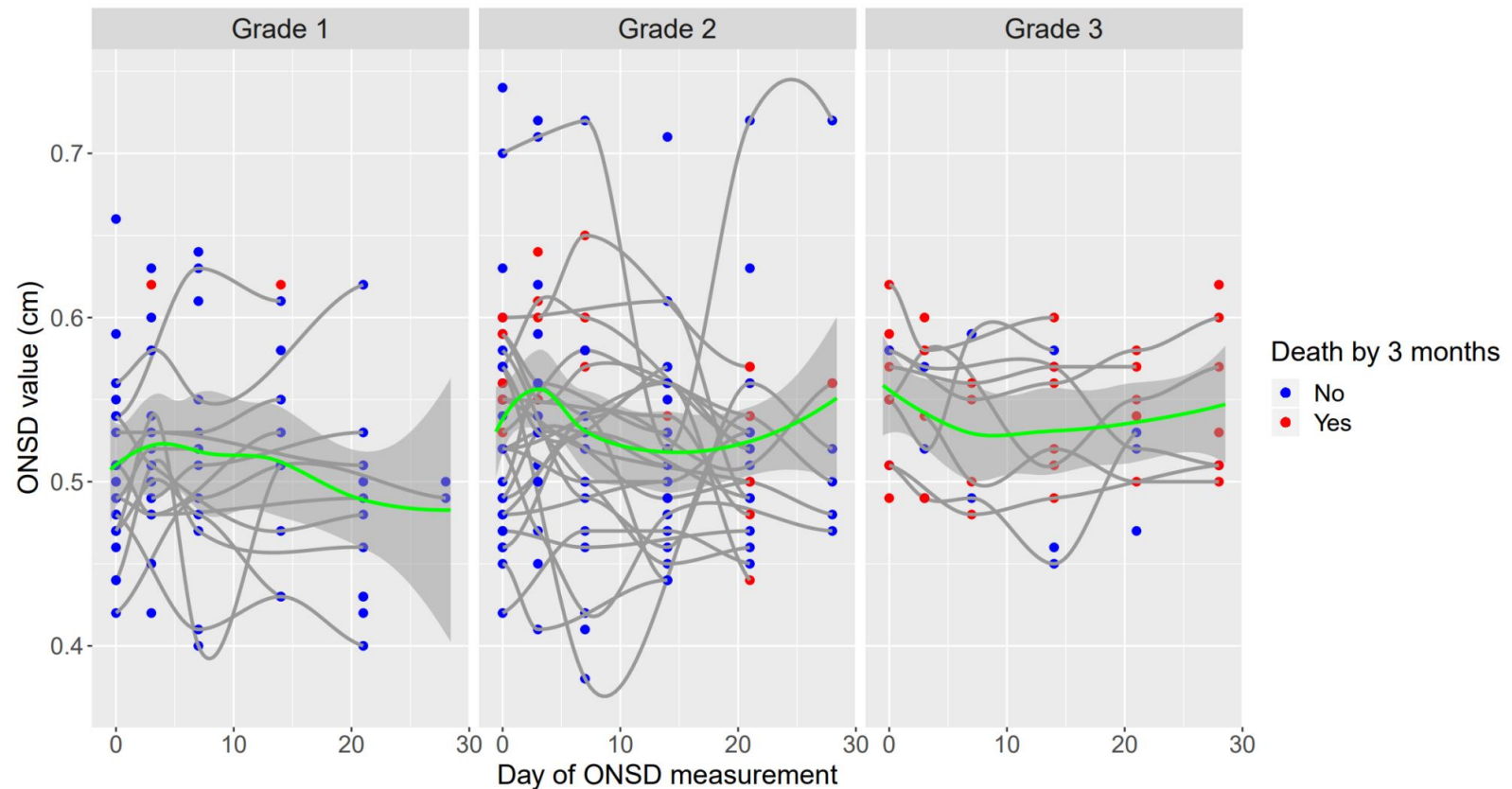
Figure 4-12: ROC curve plotting true positive rate (sensitivity) and false positive rate (1-specificity) for ONSD as a predictor of death by 3 months



ONSD=optic nerve sheath diameter. ROC=receiver operating characteristic

Higher ONSD values were observed in those with more severe disease (figure 4-13). In participants with grade 1 TBM, ONSD increases but then returns to baseline, consistent with ongoing recovery from TBM. In grade 2 and grade 3 TBM, ONSD continues to trend higher by 30 days, consistent with more severe disease in these groups.

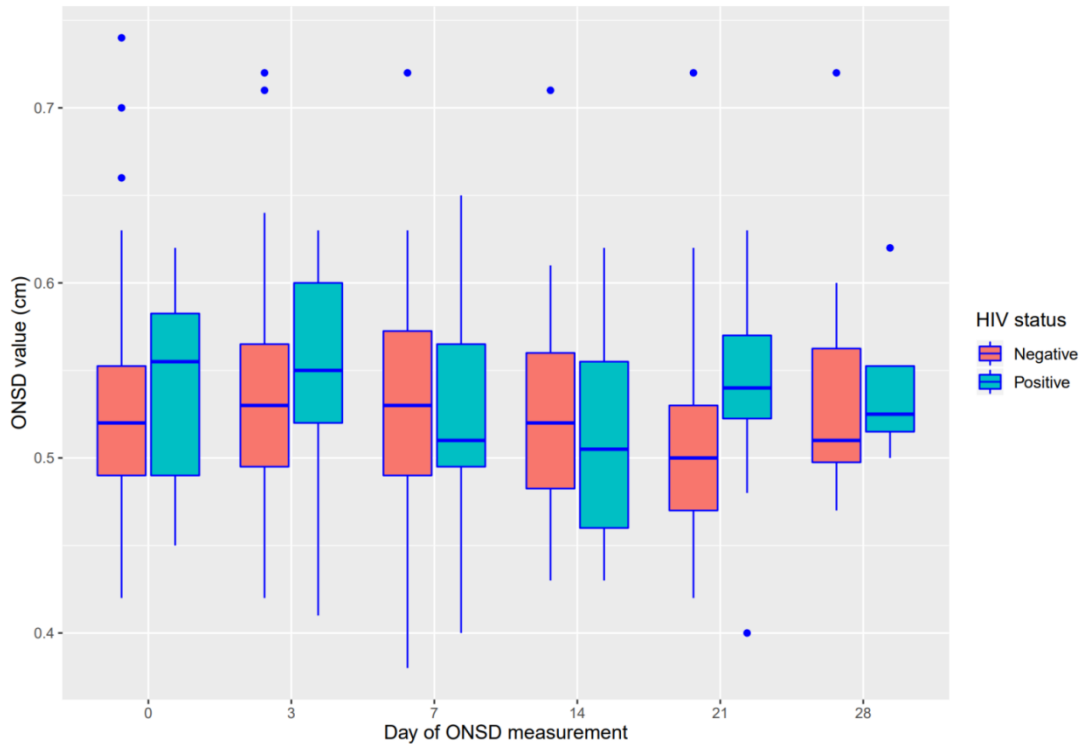
Figure 4-13: ONSD values over 30 days of anti-TB chemotherapy, stratified by TBM severity grade and death by 3 months



Individual data points represent individual ONSD values at a specified day of measurement. Data are shown stratified by grade 1, 2 and 3, where grade refers to MRC TBM severity grade. Blue dots represent ONSD values in patients who were alive by 3 months, whereas red dots represent ONSD values in patients who died by 3 months. The mean ONSD value across time points, for each grade, is represented by a green line with associated 95% CI. CI=confidence interval. MRC=Medical Research Council. ONSD=optic nerve sheath diameter. TBM=tuberculous meningitis.

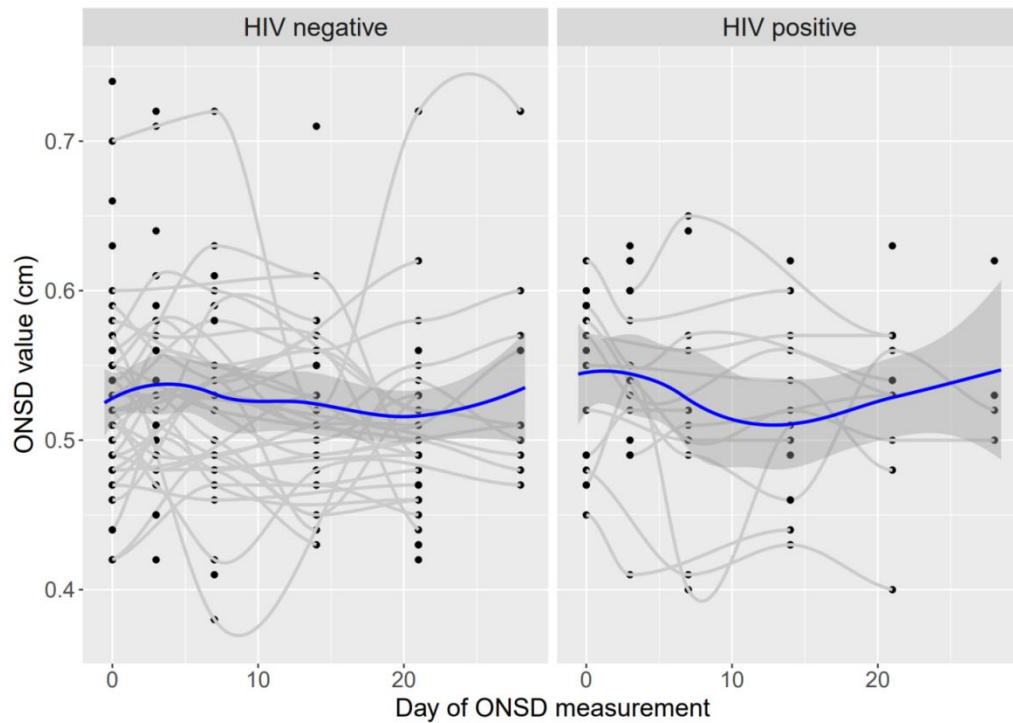
ONSD stratified by HIV over the first 30 days of anti-TB chemotherapy is shown in figures 4-14 and 4-15. ONSD was generally higher in HIV co-infection (non-significantly so) during early treatment, falling to a similar value (in those who survive), before rising again towards the end of the first month.

Figure 4-14: Boxplot over time of ONSD for all patients stratified by HIV



For each individual boxplot, the central horizontal bar represents the median value. The box contains data between 3rd quartile (upper end of box) and 1st quartile (lower end of box). Vertical lines above and below each box extend to the most extreme data point that is within 1.5x the vertical height of the box. Dots represent individual data points outside of these limits. HIV=human immunodeficiency virus. ONSD=optic nerve sheath diameter.

Figure 4-15: Scatterplot over time of ONSD for all patients stratified by HIV



Individual data points represent individual ONSD values at a specified day of measurement. Data are shown stratified by grade 1, 2 and 3, where grade refers to MRC TBM severity grade. The central bar represents the mean ONSD value (with 95% CI) across time points. CI=confidence interval. HIV=human immunodeficiency virus. ONSD=optic nerve sheath diameter.

4.4 Discussion

A high rate of neurological disability and death result from TBM, often secondary to neurological complications that act to raise ICP. Once compensatory mechanisms have been overwhelmed, neurological decline and coma result; after this critical point patient outcomes are especially poor. More evidence guiding the best detection and management of raised ICP in TBM is required. Rapid identification of raised ICP may allow earlier intervention, with potentially improved clinical outcomes. Therefore, point of care tools such as ONSD ultrasound may aid raised ICP detection in TBM, and guide management.

In this study ONSD was significantly higher in more severe presenting disease, in participants with abnormal brain imaging compared with participants with normal brain imaging, and in participants who then died by 3 months. Neurocomplications of TBM, such as hydrocephalus, tuberculomas, and paradoxical neuroinflammatory reactions, may elevate ICP. The number of participants for

whom changes consistent with raised ICP were noted on baseline brain imaging was small; and likely why ONSD did not significantly correlate with brain imaging suggestive of raised ICP. In this study median ONSD was significantly higher in participants with brain imaging consistent with neurocomplications of TBM, compared with in participants with brain imaging reported as normal. When CSF parameters were compared between these abnormal and normal brain imaging groups, significantly increased median CSF neutrophil percentage and lactate, and a significantly reduced CSF/glucose ratio, were seen in the abnormal brain imaging group vs. the normal brain imaging group. This worse CSF profile in the abnormal brain imaging represents more inflammation, and more severe disease. It appears likely that the higher ONSD values in the abnormal brain imaging group are capturing this more severe disease, with these changes manifest in changes in optic nerve size.

Additionally, baseline ONSD was significantly higher in participants who died by 3 months compared with participants who survived. This suggests that ONSD is elevated in participants with brain pathology that produces worse clinical outcomes. This correlation between ONSD and severe TBM disease leading to poor outcomes illustrates the potential value of ONSD in the management of TBM. Previous data have correlated ONSD and other proxy markers of raised ICP;[78] however our study is the first to associate higher ONSD with worse outcomes in TBM. In clinical practice, ONSD ultrasound therefore has the potential to enable earlier identification of neuroinflammatory complications that may progress to death, allowing prompt investigation and management. Using ROC curve analysis, we identified a 'best' ONSD cut-off value of 0.53cm to separate participants who died by 3 months from those who survived by 3 months.

Hyponatraemia is commonly found with TBM, although the reasons are poorly understood. Low plasma sodium predicts death in HIV co-infection.[35] Sodium-association and 30-day trend data cannot link ONSD values and plasma sodium. Low plasma sodium contributes to cerebral oedema, as the reduced oncotic pressure leads to fluid leak into the brain, resulting in raised ICP; sodium in TBM is discussed further elsewhere in this PhD.

We postulate that the future of ONSD ultrasound may be in the identification of patients at risk of neurological complications or death, and the initiation of earlier 3D brain imaging that would otherwise have not been performed. However, further work is required to build on this preliminary data and show that ONSD ultrasound can be a practically useful technique in TBM. Critically, data must show that ONSD measurements guide patient management strategies that reduce mortality and morbidity from TBM. Optimal management of neurocritical illness in TBM is uncertain, yet some strategies have accepted benefits, even if their use does not improve outcomes in all individuals.

Surgical relief of hydrocephalus through ventriculoperitoneal shunting or endoscopic third ventriculostomy may be lifesaving. Neuroinflammatory changes may respond to corticosteroids or other host directed adjunctive anti-inflammatory therapy. One approach therefore would be to use a high ONSD value as a trigger to perform 3D brain imaging. Brain imaging can be expected to identify the likely cause of elevated ONSD in TBM, and best management can proceed from there.

Evidence that ONSD reduces after administration of ICP-reducing adjunctive therapies that improve outcome would be valuable. Further research questions include what ONSD cut-off should initiate 3D brain imaging, should an ONSD cut-off vary by population or resource availability, and how frequently should ONSD ultrasound be performed in order to identify high risk patients at a stage where changes in management improve clinical outcomes?

This study has limitations. It was not possible to perform ONSD ultrasound at every time point for each participant, due to constraints on operator, participants and resources. Most scans were performed by a single operator. Whilst this allowed for consistency in scanning technique and reduced inter-operator variability, it also meant measurement could not be reviewed by a second operator. ONSD ultrasound itself has limitations; lacking standardisation of technique and value interpretation. Additionally, ONSD appearances apparently consistent with raised ICP may in fact reflect non-raised ICP pathology. Solid thickening of the optic nerve sheath (for example occurring secondary to ophthalmopathy of Graves' disease or an optic nerve sheath meningioma), or severe orbital congestion (for example occurring secondary to an arteriovenous fistula), may produce confounding appearances.[255] However, these findings are rare.

A further limitation is that ONSD was compared with brain imaging performed within 72 hours. Changes in ICP may have occurred in between ONSD ultrasound and brain imaging, reducing correlation between these two scanning modalities. The number of participants for whom radiological changes consistent with raised ICP were noted on baseline brain imaging was small, and likely why ONSD did not significantly correlate with brain imaging labelled as 'raised ICP'.

Finally, it is not known if participants received corticosteroids. Participants in this study were enrolled into one of two randomised double blinded placebo-controlled trials of adjunctive corticosteroid therapy in TBM, and the dexamethasone/placebo allocation remains unknown. Baseline data including baseline ONSD ultrasound and 3D brain imaging, prior to dexamethasone or placebo administration, were unaffected by this. Neurological complications and death by 3 months are affected by dexamethasone; an improving individual patient ONSD trend may reflect dexamethasone use if ICP was raised due to a dexamethasone-responsive cause such as

neuroinflammation. However, this blinded allocation should not affect the ability of ONSD to monitor and chart this trend.

A strength of this study is that study data was collected as part of two clinical trials with study protocols, standard operating procedures, and careful conduct of research. This is the largest study to date of ONSD ultrasound in TBM, combining longitudinal ONSD data in individual participants with clinical endpoints. Brain imaging was independently reported by an experienced neuroradiologist, and correlated with CSF parameters, which reflect measurements of inflammation at the site of disease, rather than correlation with blood parameters.

In conclusion this study demonstrated that higher ONSD values correlated with an increase disease severity, brain imaging abnormalities consistent with TBM, and an increased risk of death by 3 months, and that higher median ONSD correlated with abnormal brain imaging. ONSD ultrasound has potential for use as a bedside tool for ICP monitoring in TBM. Further research is required to understand how to harness its potential, and implementation strategies are required to translate this evidence into improved patient care.

4.5 Publications related to this chapter

The methods described within this study are published in the research protocols below:

1. Adjunctive dexamethasone for the treatment of HIV-uninfected adults with tuberculous meningitis stratified by *Leukotriene A4 hydrolase* genotype (LAST ACT): Study protocol for a randomised double blind placebo controlled non-inferiority trial

Joseph Donovan, Nguyen Hoan Phu, Le Thi Phuong Thao, Nguyen Huu Lan, Nguyen Thi Hoang Mai, Nguyen Thi Mai Trang, Nguyen Thi Thu Hiep, Tran Bao Nhu, Bui Thi Bich Hanh, Vu Thi Phuong Mai, Nguyen Duc Bang, Do Chau Giang, Dang Thi Minh Ha, Jeremy Day, Nguyen TT Thuong, Nguyen Nang Vien, Ronald B. Geskus, Tran Tinh Hien, Evelyne Kestelyn, Marcel Wolbers, Nguyen Van Vinh Chau, Guy E. Thwaites

Wellcome Open Research 2018 Mar 20;3:32

2. Adjunctive dexamethasone for the treatment of HIV-infected adults with tuberculous meningitis (ACT HIV): Study protocol for a randomised controlled trial

Joseph Donovan, Nguyen Hoan Phu, Nguyen Thi Hoang Mai, Le Tien Dung, Darma Imran, Erlina Burhan, Lam Hong Bao Ngoc, Nguyen Duc Bang, Do Chau Giang, Dang Thi Minh Ha, Jeremy Day, Le Thi Phuong Thao, Nguyen TT Thuong, Nguyen Nang Vien, Ronald B. Geskus, Marcel Wolbers, Raph L. Hamers, Reinout van Crevel, Mugi Nursaya, Kartika Maharani, Tran Tinh Hien,

Kevin Baird, Nguyen Huu Lan, Evelyne Kestelyn, Nguyen Van Vinh Chau, Guy E. Thwaites
Wellcome Open Research 2018 018 Jun 20;3:31

The data contained within this chapter are under review in the following submission:

3. Optic nerve sheath ultrasound for the detection and monitoring of raised intracranial pressure in tuberculous meningitis

Joseph Donovan, Pham Kieu Nguyet Oanh, Nicholas Dobbs, Nguyen Hoan Phu, Ho Dang Trung Nghia, David Summers, Nguyen Thuy Thuong Thuong, Guy E. Thwaites

on behalf of the Vietnam ICU Translational Applications Laboratory (VITAL) investigators

Under review at the Clinical Infectious Diseases on the date of PhD submission

Chapter 5

TBM associated hyponatraemia: an observational study of cause, treatment and outcome

5.1 Introduction

5.1.1 The control of blood sodium

Sodium, the main extracellular fluid cation in humans, is important for maintaining irritability and conduction of nerve and muscle tissues, acid-base balance,[274] and fluid homeostasis. Normal homeostatic mechanisms usually ensure tight control of blood sodium; the Na⁺ K⁺ ATPase plasma membrane pump moves Na⁺ extracellularly in exchange for K⁺ moving intracellularly,[275] whilst the renin-angiotensin-aldosterone system controls sodium's renal reabsorption and excretion. A fall in blood sodium leads to renal water excretion, whereas a rise in blood sodium leads to water conservation through increasing thirst, and the action of vasopressin in the renal tubules.[274,276] Despite these homeostatic mechanisms, disease and drug therapies may elevate or reduce blood sodium, with a variety of clinical manifestations depending on the amount and rate of change.

5.1.2 Clinical hyponatraemia

Hyponatraemia, defined as a blood sodium concentration of < 135mmol/L, is the most common electrolyte disorder in medical practice.[277] European clinical practice guidelines define hyponatraemia as mild (130-135 mmol/L), moderate (125-129 mmol/L) or profound (<125mmol/L).[277] When blood sodium falls in conjunction with reduced serum osmolality, as is usually the case, an osmotic gradient is created across cells in the brain. Water freely diffuses down this gradient and into cells, increasing cellular size[278] and resulting in oedema. The brain adapts to this oedema by reducing the number of intracellular osmotically active particles, a process which takes 24-48 hours.[277] However in acute hyponatraemia, where blood sodium falls over 48 hours or less, the brain is unable to compensate at the necessary rate, manifesting clinically as nausea, vomiting, headache, and confusion, followed by seizures, coma, and death.[277–279]

Chronic hyponatraemia presents differently to the acute form; typical presentation is of fatigue, disorientation, cognitive and gait deficits, and falls.[277–279] Caution is required when administering sodium in chronic hyponatraemia. Too rapid a correction of chronic hyponatraemia may precipitate central pontine myelinolysis, also termed the 'osmotic demyelination syndrome', a

neurological syndrome strongly associated with correction of sodium by greater than 12 mmol/L per day.[280]

5.1.3 Aetiology of hyponatraemia

Identifying the cause of hyponatraemia allows administration of correct treatment. However, defining the cause depends upon the measurement of multiple parameters, exclusion of confounding pathologies, and accurate interpretation of these data. Diagnostic flow charts, such as that in the European clinical practice guidelines,[277] rule in or out causes based upon key values such as urinary sodium and clinical assessment of fluid balance. In addition, diagnosis is supported by magnitude of blood sodium reduction, serum and urinary osmolalities, exclusion of endocrine pathologies (hypocortisolaemia and hypothyroidism), plus consideration of sodium-affecting treatments such as hypertonic saline or diuretics.

5.1.4 Hyponatraemia in tuberculous meningitis

Hyponatraemia is a common feature of TBM; in fact hyponatraemia may occur more frequently in TBM than in brain infection of different aetiology.[116] In a recent study of hyponatraemia in TBM, hyponatraemia (defined as blood sodium < 135mEq/l, twice, 24 hours apart) was identified in 34 (45%) of 76 patients.[281] Severe TBM is often associated with raised ICP due to hydrocephalus and neuro-inflammatory complications such as tuberculomas and IRIS. Brain oedema secondary to hyponatraemia may exacerbate raised ICP of TBM. In a study of patients with TBM in Vietnam, hyponatraemia was a predictor of mortality in HIV co-infected patients.[35]

Few studies have studied TBM-associated hyponatraemia. In clinical practice CSW, and SIADH are considered the most likely causes of hyponatraemia in TBM; yet few data support this.

5.1.5 Syndrome of inappropriate antidiuretic hormone secretion

SIADH results from an increase in body water coupled with an inability to adequately dilute urine and create a compensatory diuresis.[282] Plasma volume expansion results in a compensatory natriuresis to preserve volume, yet this exacerbates hyponatraemia.[282] Excessive body water accumulation may occur through inappropriate regulation of thirst, excessive oral intake by the patient independent of thirst (polydipsia), through iatrogenic administration of intravenous fluid, or anti-diuretic hormone (ADH) (also termed 'vasopressin') release from the hypothalamus by mechanisms which remain unclear.[282]

Given not all individuals with SIADH have elevated circulating levels of vasopressin, SIADH is also often referred to as the syndrome of inappropriate diuresis (SIAD).[283] A landmark study by

Zerbe et al in 1980 found and described four patterns of vasopressin release in SIADH; erratic, reset osmostat, vasopressin leak, and hypovasopressinemic antidiuresis, patterns that did not appear specific for individual diseases processes.[284] SIADH can now be classified as one of four types; A, B, C and D, each with a different mechanism; these are further described in table 5-1.

Table 5-1: Types of SIADH

Type of SIADH	Frequency of occurrence	Features
A	The most common type; occurring in 30-70% cases	Excessive and random secretion of vasopressin, with high and fixed urine osmolality, most typically seen in lung and nasopharyngeal cancers.[282,284] High vasopressin levels appear unresponsive to water intake which makes severe hyponatraemia a particular risk. Vasopressin secretion continues independent of fluid intake.[285]
B	Occurs in 20-40% cases	Vasopressin secretion at a lower osmolality than normal; termed 'vasopressin leak'. Urine osmolality is often fixed but at a lower level than type A, and protection from severe hyponatraemia occurs when further lowering of serum osmolality suppresses vasopressin.[282,284]
C	A rare cause	Failure to suppress vasopressin at low serum osmolality, possibly due to dysfunction of inhibitory neurons in the hypothalamus. During the correction of hyponatraemia vasopressin levels begin to rise inappropriately before blood sodium is corrected. This osmoregulatory defect is usually termed 'reset osmostat'. [282,284]
D	A very rare cause	Gain-of-function mutation at the V2 receptor where vasopressin acts, leads to anti-diuresis despite undetectable vasopressin and normal osmoregulation of vasopressin.[282,284,285]

SIADH can be classified as one of four types; A, B, C and D, each with a different mechanism. The terms antidiuretic hormone and vasopressin can be used interchangeably. SIADH=syndrome of inappropriate antidiuretic hormone secretion.

5.1.5.1 Diagnosis of SIADH

SIADH is suspected when blood sodium and serum osmolality are low, and urine osmolality is inappropriately high. There is continued renal excretion of sodium, an absence of clinical volume depletion, and normal renal and adrenal function, as described by Bartter and Schwartz in 1967.[286] In clinical practice guidelines for hyponatraemia[277,287] SIADH diagnostic criteria closely resemble those used in 1967 (table 5-2).

Table 5-2: Diagnostic criteria for SIADH

Essential criteria	Supplemental criteria
Effective serum osmolality <275 mOsm/kg	Serum uric acid <0.24 mmol/l (<4 mg/dl)
Urine osmolality >100 mOsm/kg at some level of decreased effective osmolality	Serum urea <3.6 mmol/L (<21.6 mg/dl)
Clinical euvolaemia	Failure to correct hyponatraemia after 0.9% saline infusion
Urine sodium concentration >30 mmol/L with normal dietary salt and water intake	Fractional sodium excretion >0.5%
Absence of adrenal, thyroid, pituitary or renal insufficiency	Fractional urea excretion >55%
No recent use of diuretic agents	Fractional uric acid excretion >12%
	Correction of hyponatraemia through fluid restriction

SIADH=syndrome of inappropriate antidiuretic hormone secretion

5.1.6 Cerebral salt wasting

CSW, defined as renal sodium loss with accompanying reduced extracellular fluid volume during intracranial disease[156] is poorly understood. First described in 1950 by Peters et al,[288] in three individuals with intracranial pathology, its very existence has been doubted.[289] The trigger to excrete large amounts of urinary sodium, a typical feature of CSW, is uncertain.[290]

5.1.6.1 Natriuretic peptides

Natriuretic peptides, particularly ANP and BNP, may be involved in pathogenesis, and roles for C-type natriuretic peptide (CNP) and dendroaspis natriuretic peptide (DNP) have also been suggested.[290] ANP and BNP are predominantly produced by the atrial and ventricular walls of the heart respectively, with additional lower level production in the brain.[291]

Whether a TBM brain insult results in increased natriuretic peptide production, or increased secretion either by the brain or the heart, is not known. Both ANP and BNP have natriuretic and aldosterone-inhibiting properties.[291] Both act in the brain to decrease salt appetite, water intake and corticotrophin release. The ANP receptor is predominantly located adjacent to the third ventricle of the brain, in an area not separated from the blood by the blood–brain barrier, and ANP binding at this site affects salt appetite and water drinking.[157] Low brain ANP levels make it unlikely that ANP actions at this site lead to CSW, although actions in the CNS may influence cardiac secretion of ANP.[156] BNP receptors are found in the hypothalamus, where BNP binding inhibits vasopressin secretion.[157] Conditions such as congestive heart failure, where BNP is elevated, are not strongly associated with hyponatraemia.[290]

Receptors for both ANP and BNP are also present in the kidneys.[157] Both ANP and BNP act in the kidney to decrease aldosterone and renin, resulting in renal sodium loss.[157] The actual mechanism of CSW may involve both hormonal mechanisms (ANP released from atrial muscle after atrial stretch with downregulation of the renin-angiotensin axis), or direct effects on neural connections to the kidneys, where interruption of sympathetic stimulation leads to increased renal blood flow and natriuresis.[156,290]

5.1.6.2 Diagnosis of CSW

CSW is characterised by hyponatraemia, hypovolaemia and inappropriately high urinary sodium and urine output. Differentiating CSW from SIADH is challenging given the key parameter to distinguish it from SIADH; extracellular fluid status, is a highly challenging assessment criterion.[290] No strict diagnostic criteria exist for CSW, and this is often a diagnosis of exclusion. Hypovolaemia may require aggressive fluid replacement and infusion of vasopressor agents such as noradrenaline. Brain ischaemia is a risk of hypovolaemia induced brain hypoperfusion, whereas overcorrection of fluid balance may result in cerebral oedema.

5.1.6.3 Assessment of extracellular fluid

Assessing a patient's extracellular volume is difficult.[292] Assessment depends on clinical history and clinical assessment, and clinical assessment alone may be inaccurate. In clinical practice, intravascular volume is frequently used as a proxy for extracellular fluid, despite it making up only

a small proportion of the extracellular fluid compartment (the majority is interstitial fluid). Gold standard measurement of intravascular volume is by radioisotopic volume measurement; unfortunately this is costly, time-consuming and not practical in most settings.[293]

5.1.6.4 Assessment of intravascular volume

5.1.6.4.1 Clinical signs of dehydration

Conventional clinical signs for identifying dehydration or reduced intravascular volume are limited. A study of 58 hyponatraemic patients (blood sodium < 130mmol/L) compared clinical assessment of extracellular fluid against 'hypovolaemia' and 'euvolaemia' diagnoses as measured by response to intravenous saline therapy.[294] Patients predicted to be 'saline responders' met at least 2 of the following 6 criteria for hypovolaemia; 1) a history or clinical setting consistent with extracellular fluid loss, 2) decreased skin turgor, axillary moisture, dry mucous membranes, or thirst, 3) greater than 0.5kg weight loss, 4) $\geq 10\%$ decrease in orthostatic blood pressure, 5) $\geq 10\%$ increase in orthostatic pulse rate, 6) Urea:Cr ratio > 20.[294] However, a saline response was only seen in 7 (24%) of 29 patients predicted by this hypovolaemia assessment. Of the 29 patients predicted to not be saline responsive, 21 (72%) of 29 patients were indeed saline non responsive.[294] In a study of 32 patients in whom history suggested hypovolaemia, capillary refill time was measured before and after 450ml blood transfusions.[295] Using age and sex specific upper limits of normal for capillary refill time, the sensitivity of capillary refill times for the detection of hypovolaemia in patients with abnormal orthostatic signs or with hypovolaemia were 26% (95% CI 7-50%) and 46% (18-75%). Capillary refill time alone was not sufficient for the detection of hypovolaemia. A systematic review of physical examination signs correlating with hypovolaemia found dry axillae and moist mucous membranes to be useful, but capillary refill time and poor skin turgor to have no proven diagnostic value.[296] A combination of signs was important to guide diagnosis of hypovolaemia whilst no individual clinical sign was particularly useful on its own.[296]

5.1.6.4.2 Clinical tools

Standing from a lying position causes ~10-15% blood to pool in lower extremities or the splanchnic system. Termed postural hypotension, this fall in blood pressure upon standing may occur secondary to dehydration (also in the elderly, due to drugs, or in those with autonomic dysfunction).[297] An already-reduced intravascular volume may experience a greater blood pressure reduction upon blood displacement to lower extremities. Central venous pressure (CVP) assessment may guide fluid resuscitation; however, interpreting CVP values requires understanding

of strengths and limitations of this technique.[298] However, none of these techniques consistently predicts intravascular volume and fluid requirements.

5.1.6.4.3 Use of inferior cava ultrasound as a clinical tool for fluid assessment

Given the difficulty in assessing intravascular fluid, as an aid to SIADH and CSW differentiation, point-of-care ultrasound of the inferior vena cava (IVC) may be beneficial. The IVC is a thin walled compliant vein, and its diameter varies with respiration. Using Doppler ultrasound IVC diameter can be measured at different phases of the respiratory cycle. In a spontaneously breathing patient, negative pressure within the thorax during inspiration causes blood to exit the IVC and enter the heart. The degree of IVC collapse during respiration, measured by the IVC collapsibility index, guides volume status and fluid management. In patients with a greater magnitude of hypovolaemia a greater degree of IVC collapse is expected. In mechanically ventilated patients, the positive intrathoracic pressure associated with inspiration leads to an increased volume of IVC blood as blood is partially prevented from entering the heart. Therefore, in mechanically ventilated patients the maximal IVC diameter occurs during inspiration; a reverse of the measurements in spontaneously breathing patients. However, the difference in IVC diameter between inspiration and expiration can be measured regardless of when in the respiratory cycle the maximal diameter occurs. Evidence supporting IVC diameter measurement in fluid assessment is described in appendix J.

5.1.6.4.4 Use of inferior vena cava ultrasound as a clinical tool for fluid assessment

In 2016 the United States Critical Care Society published an assessment of published evidence regarding IVC ultrasound for fluid assessment, alongside guidance.[299] Guidance recommended using IVC ultrasound in mechanically ventilated patients to assess likely fluid responsiveness, and advised a cutoff value of a 15% change in IVC diameter between inspiration and expiration to select those responsive from those non-responsive to fluid.[299] The Society was unable to make a recommendation regarding measurement of IVC in spontaneously breathing patients, although stated it could not be concluded that IVC ultrasound for assessment of fluid responsiveness was without merit in this group.[299]

In an American College of Emergency Physicians (ACEP) review of IVC ultrasound, a thorough scanning procedure was outlined.[300] Calculation of the caval index was as follows: $[(\text{IVC expiratory diameter} - \text{IVC inspiratory diameter}) / \text{IVC expiratory diameter} \times 100]$. A caval index (written as a percentage) close to 100% indicates almost complete venous collapse (and therefore marked volume depletion), whilst an index close to 0% suggests little collapse and

potential fluid overload.[300] ACEP recommendations advise that, in spontaneously ventilated patients with volume depletion, IVC variation will be greater than 50%, fluid overload is associated with a fixed and distended IVC, and an IVC diameter > 2.5cm, or fixed and distended, is thought to correlate with a CVP of at least 15mmHg.[300] It is important to note that responsiveness to fluid does not equate to reduced volume, although it is a helpful guide.

5.1.7 Distinguishing CSW from SIADH

Both CSW and SIADH present with low serum osmolality, high urinary osmolality and high urinary sodium.[301] Diagnostic algorithms for hyponatraemia include an assessment of extracellular fluid [277] (or in practice intravascular fluid), Intravascular fluid assessments are difficult to make, and clinical prediction of fluid status in individuals with hyponatraemia has limited sensitivity and specificity.[291]

Laboratory values may aid assessment of fluid status and guide differentiation between CSW and SIADH. Laboratory values favouring CSW are elevated haematocrit, elevated urea creatinine ratio and elevated serum protein, all of which suggest dehydration.[156] Serum uric acid may be low in SIADH, but low or normal in CSW,[156,290] whereas urinary uric acid excretion will be high in both conditions, normalising after correction of blood sodium in SIADH only.[301] In addition, serum osmolality is expected to be low in both CSW and SIADH, although it may be lower in SIADH.[156] Urinary volume should be significantly greater in CSW than in SIADH.[302]

Measurement of serum ANP and ADH does not appear beneficial in distinguishing between CSW and SIADH, due to difficulty in distinguishing cause from effect.[156] ADH may rise (to varying magnitudes) and cause SIADH, or rise as a response to compensate for the hypovolaemia of CSW to preserve volume (despite vasopressin secretion further exacerbating hyponatraemia). The pathophysiologies of CSW and SIADH may in fact be intertwined, both involving natriuretic peptide release. In CSW ADH secretion preserves circulating volume. This pattern of vasopressin secretion, where vasopressin cannot be completely suppressed in the presence of hypo-osmolality (due to a requirement to maintain volume) is similar to SIADH type B, and Moritz suggests that cases of CSW may have been included in the group originally labelled as type B SIADH.[303]

Response to salt and volume replacement may aid distinction between CSW and SIADH.

Hyponatraemia and hypovolaemia can be corrected with hypertonic saline, and assuming replacement can keep up with losses in the face of a continued natriuresis driven by pathology, euvoalaemia may be restored, and sodium deficits may be corrected. Stern et al suggest that to confirm CSW based upon response to treatment, salt administration must not only correct sodium

(as it would do this for both CSW and SIADH) but also promote a water diuresis as the hypovolaemic stimulus for vasopressin secretion is lost.[304]

In the only comparable study of sodium in TBM, Misra et al defined hyponatraemia as sodium < 135 mmol/L on two occasions 24 hours apart (with sodium testing alternate days until day 14, or until discharge if that were sooner).[281] Serum and urine osmolalities, and urinary sodium, were measured; however no longitudinal osmolality data were described. CSW was associated with low GCS and cerebral infarction, compared with non-CSW hyponatraemic patients. The authors distinguished CSW from SIADH using clinical and laboratory findings of dehydration, negative fluid balance and CVP (table 5-3).[281]

Table 5-3: Criteria to distinguish CSW from SIADH

Criteria used by Misra et al [281]	
CSW was considered in the presence of at least 2 out of 4 following features in a patient with hyponatraemia	SIADH was considered in the presence of at least 2 out of 4 following features in a patient with hyponatraemia
Clinical findings of hypovolemia such as hypotension, dry mucous membranes, tachycardia or postural hypotension	No signs of hypovolemia such as hypotension, dry mucous membrane, tachycardia or postural hypotension
Laboratory evidence of dehydration such as elevated hematocrit, hemoglobin, serum albumin or blood urea nitrogen	No laboratory evidence of dehydration such as elevated hematocrit, haemoglobin, serum albumin or blood urea nitrogen
Negative fluid balance as determined by intake output chart and/or weight loss	Normal or positive fluid balance with absence of weight loss
CVP < 6 cm of water	CVP > 6 cm of water

CSW=cerebral salt wasting. CVP=central venous pressure. SIADH=syndrome of inappropriate antidiuretic hormone secretion.

Causes of hyponatraemia were given as CSW (n=17), SIADH (n=3), drug induced (n=6), recurrent vomiting (n=4), nutritional (n=2) and adrenal insufficiency (hypopituitarism and Addison's disease, n=2).[281] No cases were linked to hypothyroidism. It was not clear how non-CSW non-SIADH diagnoses were reached; although the authors state that a history of vomiting, diarrhoea, and uses of carbamazepine, mannitol and glycerine were recorded.

5.1.7.1 Measurable differences between CSW and SIADH

When all literature is taken together, parameters suggesting CSW rather than SIADH are as follows: hypovolaemia (low extracellular volume, low plasma volume, low CVP, hypotension, signs and symptoms of dehydration),[156,277,290,301] laboratory values consistent with dehydration (elevated haematocrit,[156,290] elevated urea/creatinine ratio,[156,290,301] elevated serum protein,[156] higher urea,[277] higher serum potassium,[301] high uric acid excretion after sodium correction,[301] higher urinary sodium,[277,290] higher urinary volume,[277,290] and response to salt and fluid replacement).[156] Administration of furosemide and 0.9% saline worsen hyponatraemia in SIADH,[290] however these tests carry substantial risk.

5.1.7.2 Natriuretic peptides and ADH

In one prospective study of 24 patients with TBM and hydrocephalus a significant correlation was found between elevated plasma ANP and lower plasma sodium levels, although CSF ANP was undetected in all cases.[305] The authors hypothesised that patients with TBM may have hypothalamic ischaemia (via endarteritis affecting hypothalamic and basal ganglia perforating vessels), resulting in sympathetic discharge and atrial ANP release.[305] Whilst this study associates hyponatraemia and ANP, it does not distinguish between CSW and SIADH where both may involve ANP release.

In a further study of 67 patients with TBM in India, most of whom had hyponatraemia, serum ANP and BNP levels were significantly elevated at the time of hyponatraemia compared with hospital admission, although these natriuretic peptides were unable to distinguish CSW from SIADH.[306] In 1980 Smith and Godwin-Austen described three cases of TBM and hypersecretion of vasopressin, where in cases 1 and 2 plasma vasopressin levels were high for serum osmolality, in cases 1 and 2 there was biochemical response to fluid restriction, and in cases 2 and 3 there was little damage to the hypothalamus on post mortem which the authors hypothesised made vasopressin release through direct hypothalamic-vasopressin axis damage unlikely.[307]

In a study of 20 children with TBM in South Africa, Cotton et al demonstrated that in those children with laboratory values consistent with SIADH, vasopressin levels were significantly higher.[308] A range of plasma vasopressin levels were seen in SIADH and the authors ascribed this to patients with both 'excessive random secretion' and 'vasopressin leak' mechanisms of SIADH. A further study linking laboratory diagnosis of SIADH to raised ICP did not correlate with plasma or CSF ADH levels.[309]

Neuroendocrine hormone imbalance is common in TBM, although whether this is part of the pathogenesis of hypovolaemia in TBM is unknown. In a study of 115 patients with TBM in India

who underwent thorough blood pituitary hormone testing 62 (54%) had at least one hormone abnormality, and neuroimaging demonstrated hypothalamic-pituitary region abnormalities in 33 (29%), with most of these basal exudates.[310] Ten percent of patients were considered to have posterior pituitary damage, with a diagnosis of SIADH following the authors' definition. Serum ADH was not measured.

5.1.8 Research objectives

In a prospective descriptive analysis of the pathophysiology of TBM-associated hyponatraemia I describe the characteristics associated with hyponatraemia at presentation, and the association between plasma sodium, serum osmolality, urinary sodium and urinary osmolality. I discuss the cause(s) of hyponatraemia in TBM, and describe the progression of sodium and fluid parameters during the first 30 days of TBM treatment

5.2 Methods

5.2.1 Methods of patient recruitment from ACT HIV and LAST ACT trials

The study was nested within the ACT HIV (clinicaltrials.gov NCT03092817)[85] and LAST ACT (clinicaltrials.gov NCT03100786)[41] clinical trials. Participants in this study were Vietnamese adults (≥ 18 years of age) based only at HTD, Ho Chi Minh City, Vietnam. Inclusion and exclusion criteria, anti-TB chemotherapy, double blinded study drug allocation, ethical approval, and funding, for ACT HIV and LAST ACT, are described earlier in this thesis (chapter 3).

5.2.2 Clinical data

Baseline age, sex, TBM disease severity (MRC TBM grade) and HIV status were recorded. Participants were followed up for 3 months after enrolment, at which point neurological complications and survival were recorded. Neurological complications were defined as a fall in GCS of ≥ 2 points for ≥ 48 hours, a focal neurological sign, seizure, cerebellar signs, coma, or cerebral herniation.

5.2.3 Sodium parameters

5.2.3.1 Schedule

Study participants underwent measurement of plasma sodium, urinary sodium, serum osmolality, urinary osmolality, 24-hour fluid balance, and intravascular volume assessment at days 0, 3, 7, 14, and/or day 21 (+/- 1 day), in addition to day 28 (+/- 1 day) for laboratory tests and urinary output

measurement, and at day 30 (+/- 1 day) for IVC ultrasound. Measurements were timed from randomisation into ACT HIV or LAST ACT. Day 30 assessment was performed only if the patient remained in hospital at this time point. Plasma cortisol was measured at day 0 to exclude hypocortisolaemia.

5.2.3.2 Plasma and urine sodium

Plasma and urinary sodium were measured by a Cobas 6000 c501 chemistry analyser (Roche Diagnostics, Basel, Switzerland), available at the HTD laboratory. Spot urine sodium (rather than 24-hour collection) was used. Given plasma and urinary sodium may be influenced by diuretics, and administration of intravenous fluid containing sodium, the administration of hypertonic (3%) saline, 0.9% saline, mannitol, and/or furosemide in the preceding 24 hour period before a sodium measurement was also recorded.

5.2.3.3 Serum osmolality

Serum osmolality was measured by an Osmomat 3000 freezing point osmometer (Gonotec GmbH, Berlin, Germany). Serum was frozen at -80°C before transfer to Cho Ray Hospital, Ho Chi Minh City, for testing. Frozen serum samples were removed from the laboratory freezer at HTD and transported in a cool box (with ice) to Cho Ray Hospital, where they were tested on the same day. The short transfer period (approximately 10 minutes by road) was sufficient to defrost the sample, which is ready for analysis at arrival. Calculation of serum osmolality has reduced accuracy if other osmotically active substances (examples include alcohol, ketones, and mannitol) are present. Due to the chance of encountering such osmotically active substances in TBM, serum osmolality measurement (rather than calculation) was used.

5.2.3.4 Urine osmolality

Few data exist regarding storage of urine samples and subsequent measurement of osmolality. In this study urine samples were centrifuged following methods described by Sureda-Vives et al,[311] and transported to Cho Ray Hospital as per serum samples; in a cool box with ice, allowing defrosting on route. Sureda-Vives et al took fresh urine samples from 10 individuals, centrifuged them at 1000 rpm for 5 min at 4°C, and aliquoted them afterwards into 1-mL sterile tubes.[311] Frozen aliquots (-21°C) were then thawed in a 37°C water bath before being assayed and mixed. Frozen urine samples did not show any significant change in osmolality for up to 14 days.[311] In this study urinary osmolality was measured by an Osmomat 3000 freezing point osmometer (Gonotec GmbH, Berlin, Germany).

5.2.3.5 Limitations of serum and urine osmolality

5.2.3.5.1 Extreme values

In a retrospective cohort study of 16,598 critical care patients from an online data base in the United States, extreme osmolality (defined as $> 340\text{mmol/L}$) was associated with mortality.[312] Serum osmolality $> 400\text{mmol/L}$ is expected to be rapidly fatal. For the exclusion of erroneous high serum osmolality results a cut-off of $> 450\text{mmol/L}$ was used, where values above this threshold were excluded. Using a cut-off of $> 450\text{mmol/L}$, 4 serum osmolality values; 468, 476, 594, and 700 mmol/L , were identified and excluded from analysis.

Serum osmolality represents 2 times the sum of blood sodium and potassium, plus urea and glucose. Blood sodium must therefore be at least as low as half the serum osmolality. For the exclusion of erroneous low serum osmolality results a cut-off of $< 180\text{mmol/L}$ was used, where values below this threshold were excluded. Serum osmolality $< 180\text{mmol/L}$ would suggest plasma sodium $< 80\text{mmol/L}$ which would be incompatible with life. Using a cut-off of $< 180\text{mmol/L}$, one serum osmolality value was identified; 132 mmol/L and excluded from analysis.

5.2.3.5.2 Prolonged sample storage

Lengthy storage periods, beyond the known time thresholds of sample stability, may affect osmolality results. However, using the methods for serum and urine osmolality processing and storage described above, samples have known stability for at least 56 days (serum) and at least 14 days (urine) (appendix K). In this study, for two periods of time (each $\sim 2\text{-}3$ months) in this study, the sole method of osmolality testing became unavailable due to necessary machine repairs. During these time periods samples were stored past the evidence-based time limits and tested as soon as this testing became available again. Dates of sampling and testing were recorded. This affected only a small group of serum osmolality samples (5 samples for 3 participants). This affected 123 urine osmolality samples in 41 participants; however, most were tested within 3-4 weeks of sampling. Results from these 123 urine osmolality tests did not indicate prolonged storage led to abnormal results. No data suggest urine osmolality results are affected when samples are stored beyond these time limits following the processing steps used in this study, and these results were included in the final analysis.

5.2.3.6 The effect of dexamethasone on plasma sodium

The effect on the renal axis of high dose corticosteroid therapy must be considered. The side effects of dexamethasone include sodium retention, fluid retention, and potassium loss,[313] as expected

for a corticosteroid. Aldosterone and cortisol both act with equal affinity on the mineralocorticoid receptor in the distal nephron of the kidney, however the receptor is protected from the action of cortisol by 11β -hydroxysteroid dehydrogenase which converts cortisol to inactive cortisone.[314] In the context of 11β -OHSD deficiency, dexamethasone suppresses cortisol production and acts on the type II glucocorticoid receptor,[315] but there is also an action of cortisol on the mineralocorticoid receptor leading to, amongst other things, sodium retention.[316] Sodium transport effects are mediated through both the mineralocorticoid and glucocorticoid receptors, and dexamethasone, which binds preferentially to the glucocorticoid receptor, may still influence sodium transport this way.[317] An effect of dexamethasone on plasma sodium is expected to be small, however this effect cannot be known for certain until the treatment allocation is unblinded at the end of the trial.

5.2.4 Plasma cortisol

Hypocortisolaemia may result in hyponatraemia; therefore, a single plasma cortisol test at baseline was performed to exclude this cortisol deficient state. Day 0 serum samples were frozen at -20°C before transport in batches to Medic Medical Center, Ho Chi Minh City. Plasma cortisol was measured by a Cobas E602 chemistry analyser (Roche Diagnostics, Basel, Switzerland).

5.2.5 Fluid balance

Fluid balance was recorded over a 24-hour period, with measurements of fluid input and output, and a record of the date of start of the 24-hour period. Following standard ward procedures 24-hour collection begins at 6am.

5.2.5.1 Intravascular fluid assessment

5.2.5.1.1 IVC ultrasound

Intravascular volume was assessed by Doppler ultrasound of the IVC. IVC ultrasound was performed when participant, ultrasound operator, and ultrasound machine were available; as such it was not expected that IVC ultrasound would be available at all time points. IVC ultrasound was performed by one of two clinicians with training in critical care ultrasound, using an M-Turbo (Fujifilm Sonosite Ltd, Washington, United States) or Lumify (Philips, Amsterdam, Netherlands) ultrasound machine. The procedure for performing IVC ultrasound is as follows:

- 1) Ensure informed consent for performing IVC ultrasound.
Avoid scanning if abdominal trauma makes ultrasound probe position inaccessible.

- 2) Correctly position patient; patient should be lying on their back. Head-of-bed elevation should be no more than 30° (where 0° is a fully flat bed).
- 3) Use a clean linear probe (low frequency, e.g. 5-2 MHz). Select 'abdominal' scanning mode. Enter patient ID in machine; 'IVC' plus study ID plus day (D) of performing study e.g. IVC2015D14.
- 4) Select an appropriate scanning mode.
- 5) Stand in the most appropriate place for scanning (usually on patient's right side, level with waist) and position the screen so that it can be seen by operator.
- 6) Apply sufficient ultrasound gel to the ultrasound probe.
- 7) To obtain a sub-xiphoid view of the IVC place probe on the patient's abdomen just below the xiphoid bone with the marker facing to the head of the patient.
- 8) Adjust the probe position until the IVC can be visualised in the longitudinal plane as it enters the right atrium.
- 9) Keep IVC image on screen for period of 2-3 regular breaths.
- 10) Perform an assessment of image quality using the following criteria; a) IVC is visualised in the longitudinal plane as it enters the right atrium, b) Presence of movement artefact. If image quality is considered satisfactory then proceed to quantitative assessment of IVC.
- 11) Measure IVC diameter at maximum and minimum diameter (to allow index calculation) at a point 2cm from IVC entry into the right atrium.
- 12) Record all data on the IVC case report form.

The caval index is calculated by: $\text{IVC expiratory diameter} - \text{IVC inspiratory diameter}$, divided by $\text{IVC expiratory diameter} \times 100$. [300] Data collection additionally requires the recording of mechanical or spontaneous ventilation, and whether the patient is synchronised with their mechanical ventilation. IVC diameter measurements by ultrasound were used to allocate participants to fluid categories; 1) Expanded: IVC diameter > 2.5cm, or IVC fixed and distended [300]; 2) Reduced: >15% respiratory variation in IVC diameter (mechanical ventilation [299]) or >50% respiratory variation in IVC diameter (spontaneous ventilation [300]). Normal: assessment of IVC does not meet criteria for 'expanded' or 'reduced'. IVC maximum and IVC minimum were each recorded twice, and two caval index values were calculated. An average caval index value was then calculated for each patient.

5.2.5.2 Clinical assessment

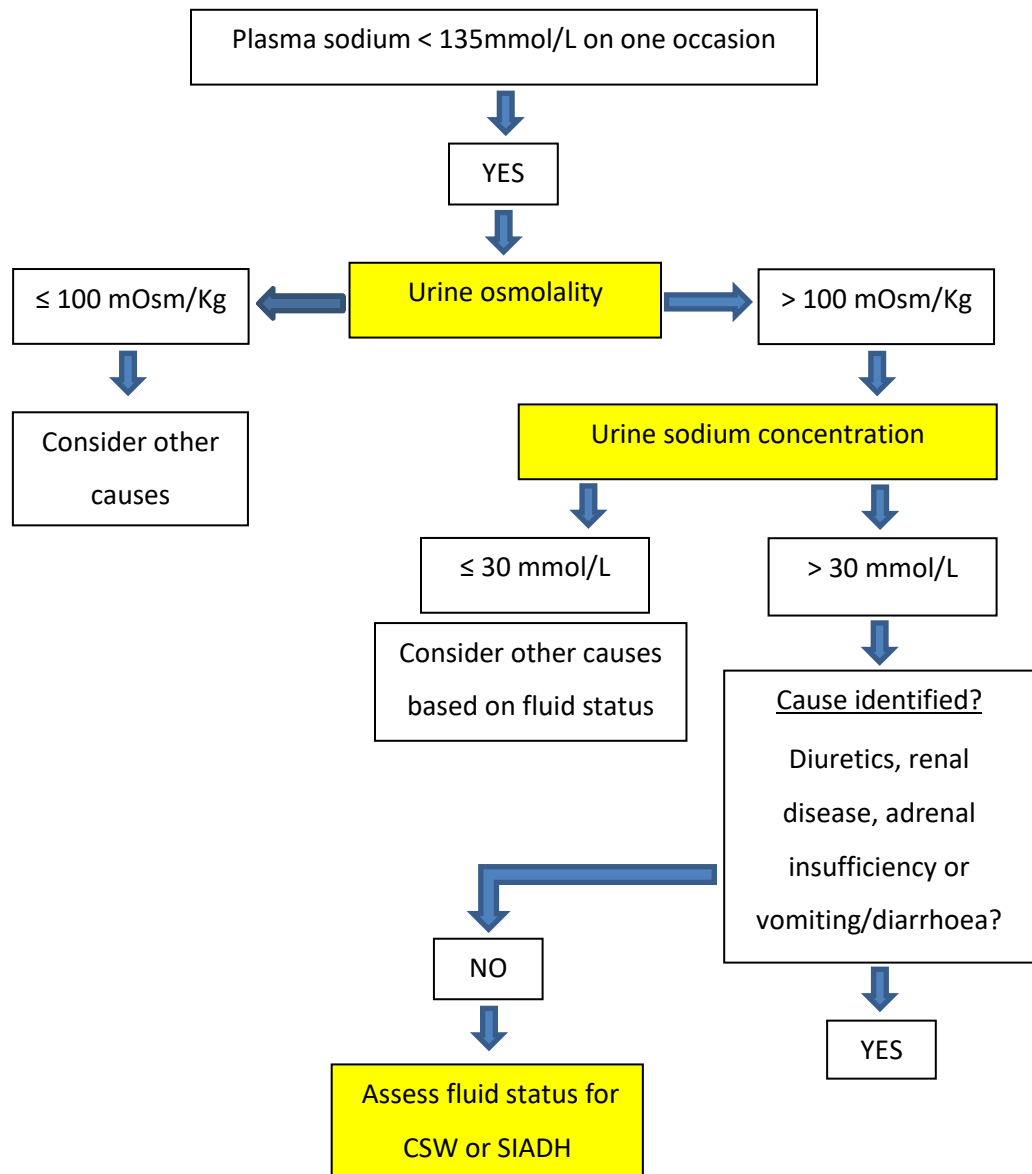
Fluid responsiveness may not always be consistent with extravascular volume status. Therefore, a clinical assessment of extracellular fluid status was also performed, for the purpose of distinguishing hypovolaemia from euvolaemia. Signs of hypovolaemia were recorded as follows: capillary refill time > 2 seconds, decreased skin turgor, dry mucous membranes, tachycardia (heart rate >100 bpm), sunken eyes. These assessment parameters were adapted from UK National Institute for Health and Care Excellence (NICE) guidelines[318] for fluid assessment, with selection of those parameters that select between reduced volume and euvolaemia. Merits and limitations of clinical signs, including in combination, for the identification of dehydration or hypovolaemia, are described in the introduction to this chapter.

In addition to IVC size and clinical assessment, laboratory parameters (elevated baseline haemoglobin, haematocrit, and urea), 24-hour fluid balance, and urinary output, were used in the assignment of causality of hyponatraemia, as part of an algorithm (table 5-4) described further below.

5.2.6 Assigning cause of hyponatraemia

Causation of hyponatraemia was initially assessed using a flowchart adapted from the European Journal of Endocrinology (EJE) 2014 hyponatraemia guidelines,[277] (figure 5-1) with participants with suspected CSW or SIADH then separated using this study's criteria (table 5-4).

Figure 5-1: Diagnostic flowchart for hyponatraemia



Flow chart adapted from European Journal of Endocrinology Clinical Practice Guidelines on Diagnosis and Treatment of Hyponatraemia.[277] CSW=cerebral salt wasting. SIADH=syndrome of inappropriate antidiuretic hormone secretion.

Figure 5-1 lists diuretics, renal disease, adrenal insufficiency or vomiting/diarrhoea, as non-CSW non-SIADH causes of hyponatraemia. Diuretic use was defined by furosemide (20mg or 40mg dosing) in the 24 hours preceding plasma sodium measurement, followed by new hyponatraemia (where ‘hyponatraemia’ was defined as plasma sodium reducing by $\geq 5\text{mmol/L}$). Renal disease was

defined by creatinine > 110 µmol/L (males) or creatinine > 90 µmol/L (females) in the 24 hours preceding plasma sodium measurement. Adrenal insufficiency was defined by a morning plasma cortisol of < 6 mcg/dL. Vomiting and/or diarrhoea was defined as the recording as an adverse event of one these in the 24-hour period prior to plasma sodium measurement. Whilst vomiting and diarrhea may occur unreported as an adverse event (low grade, or present since before commencement of study drug); episodes severe enough to alter electrolyte balance were likely to have been recorded. Hypothyroidism is a rare cause of hyponatraemia without a strong evidence base, and clinically significant hyponatraemia may only occur in severe hypothyroidism.[319] Thyroid function tests were not measured in this study.

5.2.6.1 Assigning CSW or SIADH

Assigning causality of hyponatraemia is problematic, requiring wide ranging data and often-challenging extracellular fluid assessment. In this study, assigning diagnoses of CSW and SIADH required essential information; plasma sodium < 135 mmol/l, urine sodium > 30 mmol/l, urine osmolality > 100 mOsm/kg, and no additional cause identified, plus the presence or absence of 2 of 5 criteria indicating hypovolaemia (CSW) or euvolaemia (SIADH). Using published literature and existing diagnostic criteria,[156,277,281,290,301] parameters that distinguish CSW from SIADH, and that were measurable and recordable within the hyponatraemia study of ACT HIV and LAST ACT, were identified. These parameters were used to define five criteria assessing hypovolaemia; clinical, laboratory, fluid balance, ultrasound, and urinary output, thereby differentiating CSW from SIADH. The presence of a result for each criterion scored 1 point for either CSW or SIADH (table 5-4, and below). Criteria were then totaled for each of CSW and SIADH. If more than 2 points were totaled for both CSW and SIADH, then the diagnosis with the highest score was assigned. In the event of neither CSW nor SIADH receiving 2 or more points, or equal totals for both CSW and SIADH, a diagnosis of 'Uncertain' was assigned. Hyponatraemia causality using these methods was assigned only at baseline. Further novel methods to define CSW and SIADH, using polyuria alone, hypovolaemia by ultrasound alone, and urinary sodium alone, were used to assign hyponatraemia causality at all time points (as described further below). Criteria used to distinguish CSW from SIADH in this study, alongside criteria used in a recent study of hyponatraemia in TBM,[281] are shown below in table 5-4.

Table 5-4: Comparison of criteria for distinguishing CSW from SIADH in this study vs. Misra data[281]

This study	Misra et al [281]
CSW was considered in the presence of at least 2 out of 5 following features in a patient with hyponatraemia	CSW was considered in the presence of at least 2 out of 4 following features in a patient with hyponatraemia
Clinical: Clinical findings of hypovolaemia: tachycardia, dry mucus membranes, delayed capillary refill time, sunken eyes, OR decreased skin turgor = 1 point	Clinical findings of hypovolemia such as hypotension, dry mucous membranes, tachycardia or postural hypotension
Laboratory: Elevated blood haemoglobin, haematocrit, OR urea * = 1 point	Laboratory evidence of dehydration such as elevated hematocrit, hemoglobin, serum albumin or blood urea nitrogen
Fluid balance: Negative fluid balance as determined by input output chart (24-hour fluid balance lower than +500mls) = 1 point	Negative fluid balance as determined by intake output chart and/or weight loss
Ultrasound: IVC variability (defined by >50% change in spontaneous ventilation, or > 18% change in mechanical ventilation), or IVC flat and under filled = 1 point	CVP < 6 cm of water
Urinary output: Polyuria (defined as urine output \geq 30mls/Kg in 24 hours)[320] = 1 point	
SIADH was considered in the presence of at least 2 out of 5 following features in a patient with hyponatraemia	SIADH was considered in the presence of at least 2 out of 4 following features in a patient with hyponatraemia
Clinical: No clinical findings of hypovolaemia: tachycardia, dry mucus membranes, delayed capillary refill time, sunken eyes, decreased skin turgor = 1 point	No signs of hypovolemia such as hypotension, dry mucous membrane, tachycardia or postural hypotension

Laboratory: No elevated haematocrit, haemoglobin, or urea * = 1 point	No laboratory evidence of dehydration such as elevated hematocrit, haemoglobin, serum albumin or blood urea nitrogen
Fluid balance: Normal or positive fluid balance as determined by input output chart (24-hour fluid balance \geq 500mls) = 1 point	Normal or positive fluid balance with absence of weight loss
Ultrasound: Absence of IVC variability (defined as absence of $>50\%$ change in spontaneous ventilation, or absence of $> 18\%$ change in mechanical ventilation), or absence of flat and under filled IVC = 1 point	CVP > 6 cm of water
Urinary output: Absence of polyuria (defined as urine output < 30 mls/Kg in 24 hours)[320] = 1 point	

This study's criteria are assigned after participants reach the point of assessing fluid status for either CSW or SIADH using the flowchart in figure 5-1. * Laboratory parameters were defined as 'elevated' if they were greater than the normal range, or defined as 'not elevated' if none were greater than the normal range. Upper limits of normal; haemoglobin: 16mg/dL, haematocrit: 46, urea: 7.5 mmol/l. CSW=cerebral salt wasting. CVP=central venous pressure. IVC=inferior vena cava. SIADH=syndrome of inappropriate antidiuretic hormone secretion

5.2.6.1.1 Clinical criterion

The presence or absence of tachycardia, dry mucous membranes, delayed capillary refill time, sunken eyes, and decreased skin turgor were recorded for participants. If any one of these clinical features were present, then 'yes' was recorded for the hypovolaemia 'clinical' criterion. If none were present, then 'yes' was recorded for euvolaemic 'clinical' criterion (assuming no oedema suggestive of fluid overload).

5.2.6.1.2 Laboratory criterion

The blood values of haemoglobin, haematocrit, and urea were recorded at baseline. If any one of these parameters were elevated above the normal range (upper limits of normal; haemoglobin: 16mg/dL, haematocrit: 46, urea: 7.5 mmol/l, with identical ranges used for both males and females) then 'yes' was recorded for the hypovolaemic 'laboratory' criterion. If none of these laboratory

parameters were elevated above the normal range then 'yes' was recorded for the euvolaemic 'laboratory' criterion.

5.2.6.1.3 Fluid balance criterion

Measurements of fluid input and output in a 24-hour period were recorded, and the fluid balance calculated. If fluid balance was negative, factoring in insensible losses (i.e. a value lower than +500mls in 24 hours), then 'yes' was recorded for the hypovolaemia 'fluid balance' criterion. If fluid balance was positive, factoring in insensible losses (i.e. greater than or equal to 500mls in 24 hours), 'yes' was recorded for the euvolaemic 'fluid balance' criterion.

5.2.6.1.4 Ultrasound criterion

Regarding the 'ultrasound' volume criterion, IVC appearances were viewed, and IVC variability calculated if measurements were available. If IVC appearances and IVC variability were suggestive of hypovolaemia (IVC variability >50% in spontaneously ventilating patients, or > 18% in mechanically ventilating patients, or if IVC variability could not be measured but IVC was flat and appeared under-filled), 'yes' was recorded for the hypovolaemic 'ultrasound' criterion. If IVC appearances and IVC variability were not suggestive of hypovolaemia (IVC variability was not >50% in spontaneously ventilating patients, nor > 18% in mechanically ventilating patients, or if IVC variability could not be calculated and IVC was not flat and did not appear under-filled), 'yes' was recorded for the euvolaemic 'ultrasound' criterion (unless ultrasound appearances suggested expanded IVC with <5% variation).

5.2.6.1.5 Urinary output criterion, and urinary sodium

Data suggest urinary output is higher in CSW compared with in SIADH,[277,290,321] however no evidence-based cut-off exists to distinguish between the two conditions. I therefore used an accepted definition of polyuria; urine output >30mls/Kg in 24 hours,[320] in these cases recording 'yes' for the hypovolaemic polyuria criterion. 'Higher' urinary sodium (for identification of CSW) does not have an accepted cut-off value and therefore was not used for the first hyponatraemia causality allocation.

5.2.6.2 Alternative methods to define CSW and SIADH

A hypovolaemic high urinary volume state is typical of the clinical syndrome recognised as CSW. Therefore, as additional novel analyses, CSW and SIADH were defined based on a polyuric urinary output alone (second novel analysis), by hypovolaemia assessed by IVC ultrasound alone (third novel analysis), and by the degree of urinary sodium (fourth novel analysis). Polyuria, and

hypovolaemia assessed by IVC ultrasound, were each defined as above. Urinary sodium \geq 100mmol/L was used to assign CSW (which is associated with greater urinary sodium loss), and urinary sodium <100mmol/L was used to allocate SIADH (hypovolaemia).

As previously described, polyuria was selected by the presence of 24-hour urinary output > 30mls/kg/day. Hypovolaemia by IVC ultrasound was defined by 'ultrasound criteria' listed above.

5.2.7 Assessing sodium-influencing treatments

Many drugs promote hyponatraemia; however, most are infrequently used in TBM at this study site. Use of sodium-containing fluids (hypertonic [3%] saline, 'normal' [0.9%] saline, Ringer's lactate), and hyponatraemia promoting drugs (e.g. furosemide) confound identification of causality of hyponatraemia. Hypertonic 3% saline has particularly widespread use in hyponatraemic individuals with TBM and focal neurological deficits or coma. Hypertonic 3% saline use will affect the trends of sodium and associated parameters. An electronic data search for details of hypertonic saline 3%, saline 0.9%, and furosemide 40mg, including fluid volume, and time and date of administration, was performed for study participants.

5.2.8 Statistical analysis

5.2.8.1 Sample size

This study was descriptive and exploratory, with no sample size calculation. As many participants as possible were recruited during the available time-window, which closed on 31st December 2019.

5.2.8.2 Statistical analysis plan

A statistical analysis plan written in advance of data analysis outlined the following:

- Evaluate median baseline plasma sodium, by HIV co-infection status and clinical characteristics including MRC TBM grade
- Establish whether there is any correlation between baseline plasma sodium, serum osmolality, urinary sodium, urinary osmolality, and plasma cortisol, in TBM
- Describe the progression of sodium and associated parameters during the first 30 days of TBM treatment
- Describe the influence of baseline plasma sodium, serum osmolality, urinary sodium, urinary osmolality, and plasma cortisol, on clinical outcomes in TBM

Subsequent to data collection, the number of baseline parameters assessed for correlation with baseline plasma sodium was expanded and defined, to include: days of symptoms, cranial nerve palsy, seizures, highest temperature ($^{\circ}\text{C}$), lumbar CSF opening pressure (cmH_2O), and baseline CSF parameters.

Additionally, at the end of data collection a decision was made to search for sodium-containing fluid treatment details, to allow description of use of these therapies.

5.2.8.3 Statistical tests

The Shapiro-Wilk test for normality was applied to data. Measured parameters (for example plasma sodium) were both normally distributed, and non-normally distributed, when assessed at individual time points (e.g. day 21). Therefore, data was treated as non-parametric data in this analysis; data are shown using medians (with IQR). Non-normally distributed data were compared by the Wilcoxon rank sum test. Correlation between continuous variables was performed using Spearman's correlation. Data were analysed using R (version 3.6).

5.3 Results

5.3.1 The study population

From June 2017 to December 2019 inclusive, 208 participants with TBM underwent sodium parameter testing, undergoing at least one test for plasma sodium, urinary sodium, serum osmolality, or urine osmolality, at 1 or more of 6 time points (days 0, 3, 7, 14, 21, and 28). The median age of the study population was 37 (IQR 29-46) years. 71.9% (153/208) participants were male. MRC TBM severity grades[9,236] amongst the study population were, Grade 1: 38.5% (n=80), Grade 2: 47.6% (n=99), Grade 3: 13.9% (n=29). 71.6% (n=149) study participants were diagnosed with definite TBM, 9.6% (n=20) with probable TBM, and 14.4% (n=30) with possible TBM. 42.8% (89/208) participants were HIV co-infected.

In total, during the first 30 days of anti-TB chemotherapy for these 208 participants the following measurements were performed: 734 plasma sodium, 407 urinary sodium, 743 serum osmolality, 728 urinary osmolality, 208 plasma cortisol, 769 fluid balance, 764 urine output, and 226 IVC ultrasound, measurements.

5.3.2 Baseline plasma sodium

Baseline plasma sodium (day 0 +/- 1 day) was available for 91.3% (190/208) participants. Median baseline plasma sodium with IQR is shown for all participants, and stratified by HIV co-infection and by MRC TBM grade, in table 5-5.

Table 5-5: Baseline plasma sodium

	No. participants	Plasma sodium (mmol/L)	P value
All participants (Median[IQR])	190	126 (122-131)	
HIV co-infection (Median[IQR])			
- Co-infected	80	125 (121-130)	0.18
- Uninfected	110	126 (122-131)	
MRC TBM severity grade (Median[IQR])			
- 1	70	128 (124-132)	0.002 #
- 2	91	125 (121-128)	
- 3	29	126 (121-132)	

Grade 1 compared with grades 2 & 3 combined. Median plasma sodium value is shown with IQR. P value represents Wilcoxon rank sum test; given plasma sodium values were non-normally distributed. HIV=human immunodeficiency virus. IQR=interquartile range. MRC=Medical Research Council. TBM=tuberculous meningitis.

5.3.3 Severity of baseline hyponatraemia

176/190 (92.6%) participants for whom baseline plasma sodium was available were hyponatraemic (defined as Na<135mmol/L) at baseline. In 134/190 (70.5%) participants baseline plasma sodium was <130mmol/L, and in 75/190 (39.5%) participants baseline plasma sodium was <125mmol/L.

Participants were separated into either mild (Na≥130, Na<135, mmol/L), moderate (Na <130, Na≥125, mmol/L) or profound (Na<125mmol/L) severity categories for hyponatraemia.[277]

Severity of hyponatraemia, stratified by HIV co-infection status and MRC TBM severity grade, is described in table 5-6.

Table 5-6: Mild, moderate, and profound severity of hyponatraemia in participants with TBM

	No.	Mild hyponatraemia (Na \geq 130, Na<135) N (%)	Moderate hyponatraemia (Na <130, Na \geq 125) N (%)	Profound hyponatraemia (Na<125mmol/L) N (%)
All participants	176	42 (23.9%)	59 (33.5%)	75 (42.6%)
HIV				
Co-infected	73	16 (21.9%)	20 (27.4%)	37 (50.7%)
Uninfected	103	26 (25.2%)	39 (37.9%)	38 (36.9%)
MRC TBM severity grade				
1	66	25 (37.9%)	23 (34.8%)	18 (27.3%)
2	88	13 (14.8%)	31 (35.2%)	44 (50.0%)
3	22	4 (18.2%)	5 (22.7%)	13 (59.1%)

Plasma sodium concentrations reported in mmol/L. HIV=human immunodeficiency virus.
MRC=Medical Research Council. TBM=tuberculous meningitis.

5.3.4 Comparison of baseline parameters by status and severity of hyponatraemia

Data describing plasma osmolality, urinary sodium, urinary osmolality, and urine output, by severity of hyponatraemia at baseline, alongside participants without hyponatraemia at baseline, are shown in table 5-7. Trends of reducing serum osmolality, increasing urinary sodium, and increasing urinary osmolality occurred as hyponatraemia severity progressed from mild to profound. Additionally, plasma cortisol levels increased with increasing severity of hyponatraemia.

Table 5-7: Baseline parameters by status and severity of hyponatraemia

	N	All participants	N	Non-hyponatraemic (Na \geq 135mmol/L)	N	Mild hyponatraemia (Na \geq 130, Na<135)	N	Moderate hyponatraemia (Na <130, Na \geq 125)	N	Profound hyponatraemia (Na<125mmol/L)
Urinary sodium (mmol/L) (Median[IQR])	135	89 (52-119)	10	84 (38-105)	29	80 (42-110)	47	86 (56-112)	49	111 (58-133)
Serum osmolality (mOsm/Kg) (Median[IQR])	146	271 (262-281)	11	289 (285-297)	34	279 (276-287)	47	268 (262-274)	54	264 (253-270)
Urinary osmolality (mOsm/Kg) (Median[IQR])	142	490 (378-630)	10	438 (397-551)	31	386 (299-596)	48	493 (400-622)	53	546 (414-654)
24-hour urinary output (mls) (Median[IQR])	151	2400 (2100-2800)	9	2600 (2300-3500)	32	2500 (2100-2750)	48	2400 (2100-2600)	62	2300 (2000-2800)
Hypovolaemic IVC (N[%])	57	16/57 (28.1%)	3	1/3 (33.3%)	15	1/15 (6.7%)	16	6/16 (37.5%)	23	8/23 (34.8%)

Plasma cortisol (mcg/dL) (Median[IQR])	190	19.8 (15.1-25.0)	14	18.8 (16.8-20.9)	42	18.5 (10.1-23.1)	59	19.3 (13.5-25.2)	75	22.2 (16.9-27.0)
Haemoglobin (g/dL) (Median[IQR])	189	12.6 (11.0-13.8)	14	12.5 (10.3-14.2)	42	13.1 (11.2-14.0)	58	12.7 (11.4-13.6)	75	12.1 (10.7-13.6)
Urea (mmol/L) (Median[IQR])	164	4.3 (3.2-5.6)	12	5.6 (4.2-10.9)	35	4.5 (3.2-5.2)	51	5.0 (3.4-5.9)	66	4.0 (3.0-5.0)

All participants with available plasma sodium at baseline are represented. Data are non-normally distributed, as such they are summarised by median and IQR. Haemoglobin and urea were measured from blood. IQR=interquartile range. IVC=inferior vena cava. N=number of participants. Na=sodium.

5.3.5 Baseline plasma sodium correlations

Correlation of baseline plasma sodium with clinical and CSF parameters is shown in table 5-8. Lower plasma sodium correlated significantly with higher lumbar CSF opening pressure, higher absolute CSF neutrophils and CSF neutrophil %, and with lower CSF/blood glucose ratio. A trend towards higher CSF lactate was seen with lower plasma sodium.

Table 5-8: Correlation of baseline plasma sodium with clinical and CSF parameters

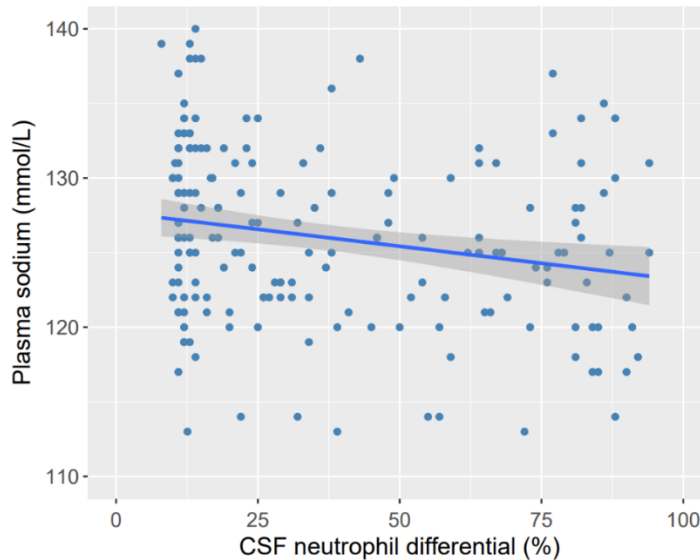
Parameter	Total No.	Correlation co-efficient	P value
Days of symptoms	135	-0.02	0.76
Cranial nerve palsy	198	NA *	0.11
Seizures	200	NA *	0.12
Glasgow coma score	187	0.11	0.14
Highest temperature (°C)	187	-0.11	0.14
Lumbar CSF opening pressure (cmH ₂ O)	111	-0.22	0.02
CSF WBC (cells/mm ³)	183	-0.12	0.12
CSF neutrophil %	181	-0.21	0.01
Absolute CSF neutrophil count (cells/mm ³)	181	-0.19	0.01
CSF/blood glucose ratio	183	0.19	0.01
CSF protein (g/L)	184	-0.04	0.57
CSF lactate (mmol/L)	184	-0.13	0.08

Correlation is shown for plasma sodium at baseline (day 0) with each listed variable in the table. Co-efficient and significance (p value) are shown using Spearman's rank correlation co-efficient.

*Comparison for binary variables (cranial nerve palsies and seizures) was performed by Wilcoxon rank sum test given plasma sodium values were non-normally distributed, therefore no correlation co-efficient is shown. Data for baseline cranial nerve palsy (n=198) reflects total of cranial nerve palsy (n=39) and no cranial nerve palsy (n=159). Data for baseline seizures (n=200) reflects total of seizures (n=8) and no seizures (n=192). CSF=cerebrospinal fluid. NA=not applicable. WBC=white blood cells.

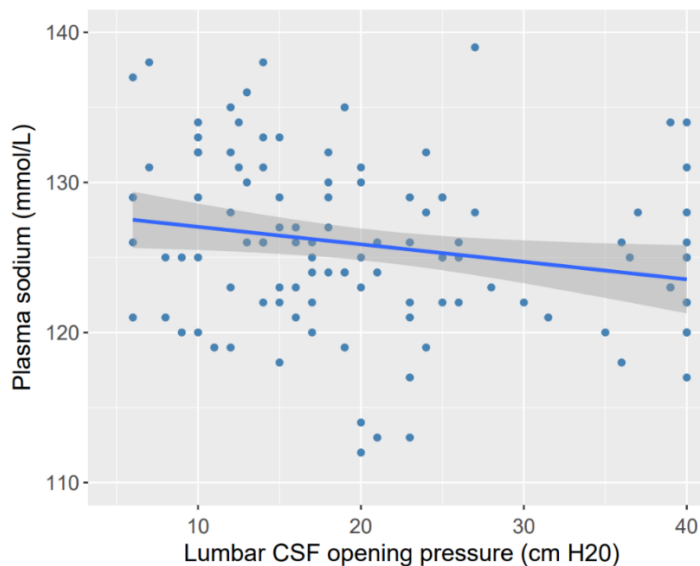
A significant negative association was seen for baseline plasma sodium and each of CSF neutrophil differential ($p=0.01$) (figure 5-2), lumbar CSF opening pressure ($p=0.02$) (figure 5-3), and absolute CSF neutrophils. A significant positive association was seen for baseline plasma sodium and CSF/blood glucose ratio (figure 5-4).

Figure 5-2: The correlation of plasma sodium and CSF neutrophil differential



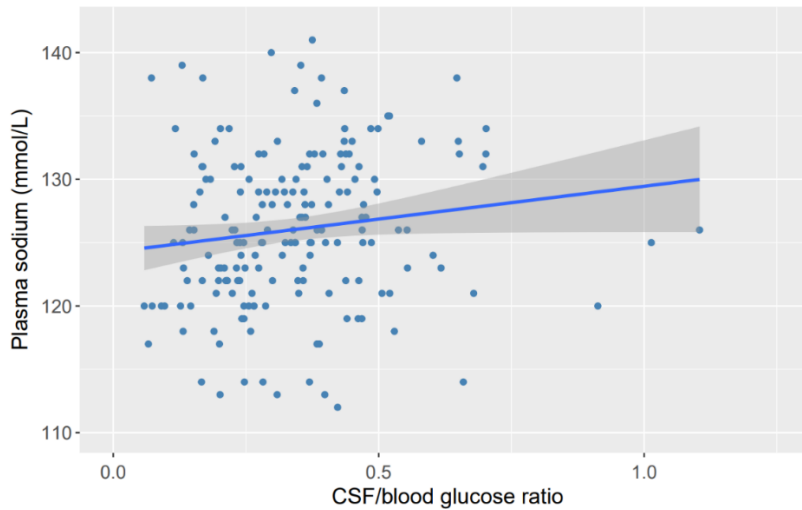
Individual points represent individual participants with both plasma sodium and CSF neutrophil measurements. A central blue line of best fit is surrounded by grey shading defined by the 95% confidence interval. CSF=cerebrospinal fluid

Figure 5-3: The correlation of plasma sodium and lumbar CSF opening pressure



Individual points represent individual participants with both plasma sodium and CSF neutrophil measurements. A central blue line of best fit is surrounded by grey shading defined by the 95% confidence interval. CSF=cerebrospinal fluid

Figure 5-4: The correlation of plasma sodium and CSF/blood glucose ratio



Individual points represent individual participants with both plasma sodium and paired CSF/blood glucose measurements. A central blue line of best fit is surrounded by grey shading defined by the 95% confidence interval. CSF=cerebrospinal fluid

To fully describe the clinical picture of hyponatraemia in TBM, an analysis of the associations of plasma sodium with other relevant parameters was performed. Correlation of baseline plasma sodium with baseline serum and fluid parameters is shown in table 5-9.

Table 5-9: Correlation of baseline plasma sodium with other baseline sodium and fluid parameters

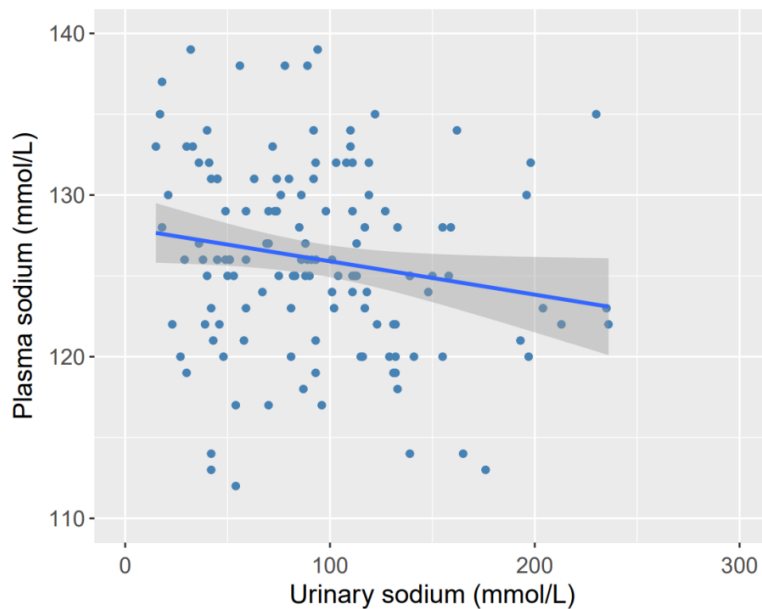
Parameter	Total No.	Correlation co-efficient	P value
Urinary sodium (mmol/L)	135	-0.20	0.02
Serum osmolality (mmol/L)	146	0.62	<0.001
Urinary osmolality (mmol/L)	142	-0.18	0.04
24-hour fluid balance	151	-0.04	0.59
24-hour fluid output	151	0.06	0.50
Baseline cortisol	190	-0.23	0.001

Correlation is shown for plasma sodium at baseline (day 0) with each listed variable in the table. Co-efficient and significance (p value) are shown using Spearman's rank correlation co-efficient. CSF=cerebrospinal fluid. WBC=white blood cells.

5.3.5.1 Baseline plasma sodium and urinary sodium

A significant correlation was seen between baseline plasma sodium and baseline urinary sodium (n=135, p=0.02) in all participants. No significant correlation was seen within HIV co-infection subgroups (HIV co-infection: n=47, correlation co-efficient -0.17, p=0.26; HIV uninfected: n=88, correlation co-efficient -0.19, p=0.08), nor with MRC TBM severity grade subgroup 1 or 3 (Grade 1: n=52, correlation co-efficient -0.01, p=0.95; Grade 3: n=18, correlation co-efficient -0.32, p=0.20). In participants with MRC TBM severity grade 2, a significant correlation between baseline plasma sodium and urinary sodium was seen (Grade 2: n=65, correlation co-efficient -0.26, p=0.03). Correlation of baseline plasma sodium and urinary sodium, for all participants, is shown in figure 5-5. In an analysis of correlation between baseline plasma sodium and baseline urinary sodium stratified by severity of hyponatraemia (mild, moderate, or profound - as defined in table 5-7), no significant correlations were seen; mild hyponatraemia: n=29, correlation co-efficient -0.02, p=0.90; moderate hyponatraemia: n=47, correlation co-efficient -0.09, p=0.54; profound hyponatraemia: n=49, correlation co-efficient 0.08, p=0.58.

Figure 5-5: Correlation of baseline plasma sodium and urinary sodium

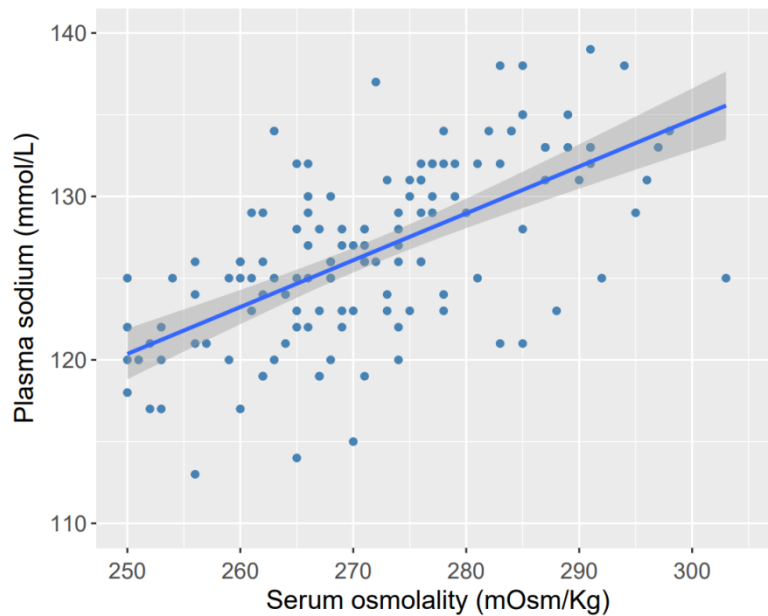


Individual points represent individual participants with both plasma sodium and urinary sodium measurements. A central blue line of best fit is surrounded by grey shading defining the 95% confidence interval.

5.3.5.2 Baseline plasma sodium and baseline serum osmolality

A positive correlation was seen between baseline plasma sodium and baseline serum osmolality (correlation co-efficient 0.6, $p < 0.001$). A further analysis of the association between baseline plasma sodium and baseline serum osmolality showed that significant positive correlation between these parameters is retained in participants with HIV co-infection ($n=47$, correlation co-efficient 0.66, $p < 0.001$) and in HIV uninfected participants ($n=99$, correlation co-efficient 0.59, $p < 0.001$). Additionally, significant correlation is retained between baseline plasma sodium and baseline serum osmolality in participants with TBM grade 2 ($n=69$, correlation co-efficient 0.53, $p < 0.001$), and in participants with TBM grade 3 ($n=20$, correlation co-efficient 0.90, $p < 0.001$), but not in participants with TBM grade 1 ($n=57$, correlation co-efficient 0.56, $p=0.86$). Correlation of plasma sodium and serum osmolality is shown for all participants in figure 5-6. In an analysis of correlation between baseline plasma sodium and baseline serum osmolality stratified by severity of hyponatraemia (mild, moderate, or profound), significant correlations were seen with mild hyponatraemia: $n=34$, correlation co-efficient 0.41, $p=0.02$; and with moderate hyponatraemia: $n=47$, correlation co-efficient 0.32, $p=0.02$. A trend towards significance was seen with profound hyponatraemia: $n=54$, correlation co-efficient 0.24, $p=0.07$.

Figure 5-6: Correlation of baseline plasma sodium and serum osmolality

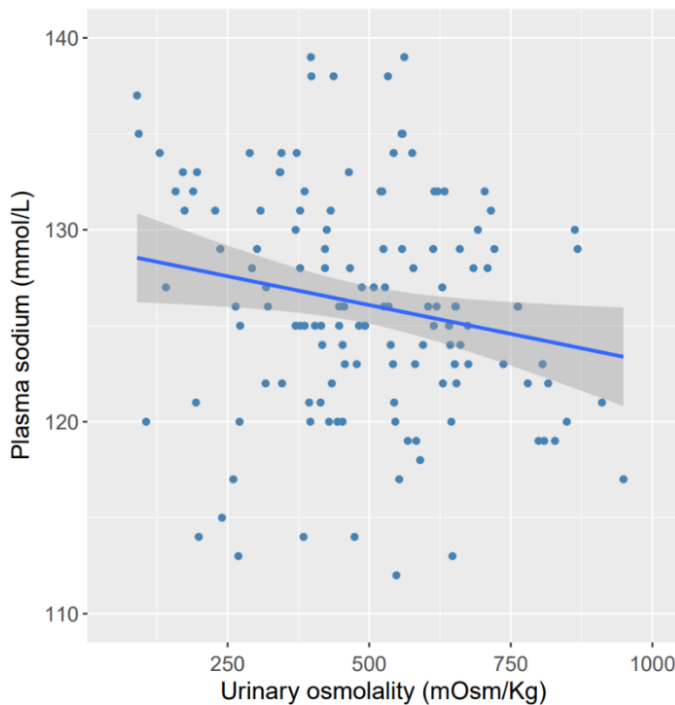


Individual points represent individual participants with both plasma sodium and serum osmolality measurements. A central blue line of best fit is surrounded by grey shading defining the 95% confidence interval. CSF=cerebrospinal fluid

5.3.5.3 Plasma sodium and urinary osmolality

A significant correlation between plasma sodium and urinary osmolality was seen for all participants (correlation co-efficient -0.18, $p=0.04$) (figure 5-7).

Figure 5-7: Correlation of baseline plasma sodium and urinary osmolality



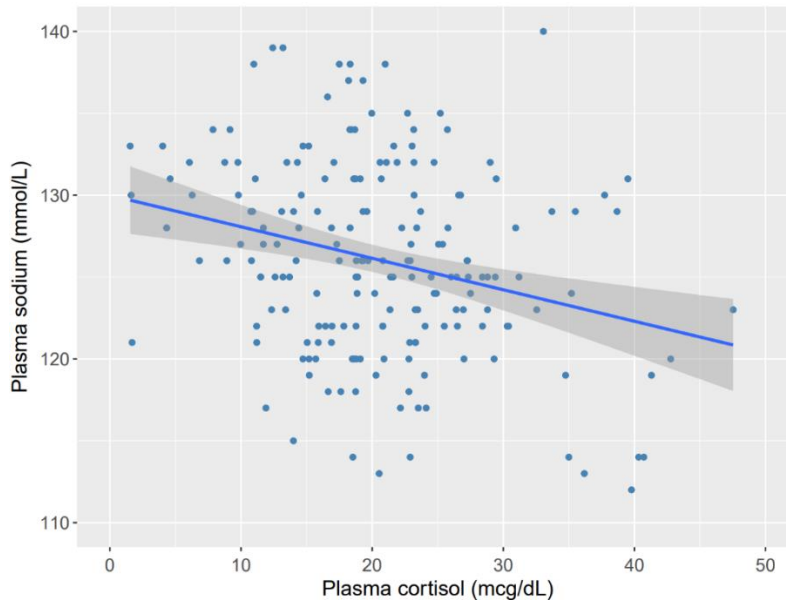
Individual points represent individual participants with both plasma sodium and urine osmolality measurements. A central blue line of best fit is surrounded by grey shading defining the 95% confidence interval.

A significant correlation between plasma sodium and urinary osmolality was seen in HIV uninfected participants ($n=98$, correlation co-efficient -0.20 , $p=0.05$), but not in HIV co-infected participants ($n=44$, correlation co-efficient -0.10 , $p=0.53$). No significance between plasma sodium and urinary osmolality was seen stratified by MRC TBM grade (grade 1: $n=56$, correlation co-efficient -0.12 , $p=0.39$; grade 2: $n=66$, correlation co-efficient -0.14 , $p=0.26$; grade 3: $n=20$, correlation co-efficient -0.18 , $p=0.45$).

5.3.5.4 Baseline plasma sodium and baseline plasma cortisol

Plasma sodium was significantly and inversely associated with plasma cortisol at baseline (correlation co-efficient -0.23 , $p=0.001$), suggesting a stress response (elevated plasma cortisol) with more severe disease (lower plasma sodium) (figure 5-8). Median baseline cortisol was higher in participants with profound hyponatraemia (median 22.2mcg/dL , IQR $16.9\text{-}27.0$) vs. participants with mild or moderate hyponatraemia (median 18.9mcg/dL , IQR $13.1\text{-}23.7$) ($p=0.01$).

Figure 5-8: Correlation of baseline plasma sodium and plasma cortisol



Individual points represent individual participants with both plasma sodium and plasma cortisol measurements. A central blue line of best fit is surrounded by grey shading defining the 95% confidence interval.

A further analysis of the association between baseline plasma sodium and baseline plasma cortisol showed that significant correlation between these parameters is retained in participants with HIV co-infection ($n=80$, correlation co-efficient -0.25 , $p=0.03$), and in HIV uninfected participants ($n=110$, correlation co-efficient -0.22 , $p=0.02$). No significant correlation between baseline plasma sodium and baseline plasma cortisol was seen stratified by MRC TBM severity grade (grade 1: $n=56$, correlation co-efficient -0.21 , $p=0.08$; grade 2: $n=66$, correlation co-efficient -0.09 , $p=0.39$; grade 3: $n=20$, correlation co-efficient -0.36 , $p=0.05$).

5.3.5.5 Non-plasma sodium baseline correlations

Urinary sodium correlated negatively with serum osmolality at baseline ($n=130$, correlation co-efficient -0.24 , $p=0.01$), and with urinary osmolality at baseline ($n=126$, correlation co-efficient 0.44 , $p<0.001$), but not with 24-hour urine output at baseline ($n=115$, correlation co-efficient 0.05 , $p=0.61$).

5.3.6 Assigning causality of hyponatraemia at baseline

5.3.6.1 This study's criteria

Of 176 hyponatraemic participants, 8 participants had urinary sodium $\leq 30\text{mmol/L}$ and could therefore not meet criteria for either CSW or SIADH. No participants had urinary osmolality $\leq 100\text{mOsm/Kg}$. Data were available for clinical (n=54), laboratory (n=176), fluid balance (n=142), IVC (n=48) and urinary output (n=142), respectively. Complete data sets assessing clinical, laboratory, fluid balance, and IVC ultrasound hypovolaemia parameters, were available for 34/168 (20.2%) hyponatraemic participants who had progressed through figure 5-1 to fluid status assessment for CSW or SIADH. In this restricted sub-group, following the criteria described above, baseline diagnoses of CSW or SIADH were allocated as follows; 7/34 (20.6%) CSW, and 27/34 (79.4%) SIADH.

Of these 34 participants, all those with mild hyponatraemia (n=9) fitted the definition for SIADH. In those with moderate hyponatraemia (n=10) there were 2 cases of CSW (20%) and 8 cases of SIADH (80%), and in those with profound hyponatraemia (n=15) there were 5 cases of CSW (33.3%) and 10 cases of SIADH (66.7%). Individual scores contributing to diagnosis allocation are shown in table 5-10. Median urinary sodium (which did not contribute towards diagnosis allocation) of each group was CSW; 85 (IQR 72-109) and SIADH; 80 (IQR 49-126).

Table 5-10: Individual scores contributing towards allocation of this study's criteria

	Number of times assessment criteria met				
All participants (N=34)	Clinical	Laboratory	Fluid balance	IVC	Polyuria
Participants with total score favouring CSW (hypovolaemia) (N=7)	5	1	3	6	7
Participants with total score favouring SIADH (euvolaemia) (N=27)	21	26	22	23	5

CSW=cerebral salt wasting. IVC=inferior vena cava. SIADH=syndrome of inappropriate anti-diuretic hormone secretion

To investigate whether this sub-population of 34 participants with complete data sets assessing clinical, laboratory, fluid balance, IVC ultrasound, and urinary output parameters, was representative of the study's baseline-hyponatraemic population (n=176), these populations were compared (table 5-11). The population assessed by this study's hyponatraemia causality criteria (n=34) appeared representative of the study's baseline-hyponatraemic population (n=176).

Table 5-11: Comparison of study participants assessed by this study's hyponatraemia causality criteria (n=34) with baseline-hyponatraemic population (n=176)

	Participants with complete dataset for fluid balance assessment (%) N=34	All hyponatraemic participants in this study (%) N=176
HIV co-infection		
- Co-infected	11 (32.4%)	73 (41.5%)
- Uninfected	23 (67.6%)	103 (58.5%)
MRC TBM severity grade		
- 1	10 (29.4%)	66 (37.5%)
- 2	20 (58.8%)	88 (50.0%)
- 3	4 (11.8%)	22 (12.5%)
Baseline plasma sodium severity		
- Mild	9 (26.5%)	42 (23.9%)
- Moderate	10 (29.4%)	55 (31.3%)
- Severe	15 (44.1%)	79 (44.9%)
Survival by 3 months		
- Alive	25 (73.5%)	133 (75.6%)
- Dead	9 (26.5%)	43 (24.4%)

HIV=human immunodeficiency virus. MRC=Medical Research Council. N=number of participants. TBM=tuberculous meningitis.

5.3.6.2 Urinary volume

As a second novel analysis of hyponatraemia causality, the presence or absence of polyuria was used to allocate CSW (polyuria) or SIADH (non-polyuria) diagnoses, consistent with the clinical phenotypes of these two conditions. Baseline data were available for analysis in 142/176 (80.7%) hyponatraemic participants. Polyuria was present in 129/142 (90.8%) participants, with non-polyuria urinary output present in 13/142 (9.2%) participants. This result differs from the first

causality analysis above (table 5-10), where 7/34 (20.6%) baseline-hyponatraemic participants were assigned a diagnosis of CSW. Polyuria, by a weight based definition, was present in most participants who were hyponatraemic at baseline, suggesting most participants may indeed have some degree of CSW (given SIADH would result in low-normal urinary volume) but not enough to impact upon clinical or laboratory criteria. The weighting given to polyuria in the first hyponatraemia causality analysis (table 5-10), i.e. 1 of 5 criteria, may be insufficient for assigning CSW, given polyuria is likely an important parameter in identifying CSW.

5.3.6.3 Intravascular volume

As a third novel analysis of hyponatraemia causality, the presence or absence of hypovolaemia by IVC ultrasound was used to allocate CSW (hypovolaemia) and SIADH (no hypovolaemia) diagnoses, again consistent with the clinical phenotypes of these two conditions. Baseline data were available for analysis for 54/176 (30.7%) hyponatraemic participants. Reduced intravascular volume, as defined in study methods, was used to allocate a diagnosis of CSW in 15/54 (27.8%) participants, with IVC ultrasound not suggestive of reduced intravascular volume in 33/54 (61.1%) participants.

5.3.6.4 Urinary sodium

As a fourth novel analysis of hyponatraemia causality, the degree of urinary sodium ($\geq 100\text{mmol/L}$, or $<100\text{mmol/L}$) was used to allocate CSW (hypovolaemia) and SIADH (no hypovolaemia) diagnoses, respectively, again consistent with the clinical phenotypes of these two conditions. Data were available for analysis for 125/176 (71.0%) hyponatraemic participants. Higher urinary sodium, as defined in study methods, was used to allocate CSW in 72/125 (57.6%) participants, with IVC ultrasound not suggestive of reduced intravascular volume in 53/125 (42.4%) participants.

5.3.7 Re-assigning causality of hyponatraemia during the first 30 days of anti-tuberculous chemotherapy

Causality of hyponatraemia was reassigned at each of day 3, 7, 14, 21, and 28 based on parameters available at each of these time points. Assessments were performed using urinary volume, intravascular volume (ultrasound), and urinary sodium methods, given lack of laboratory data (required for this study's criteria) at these time points. These diagnosis allocations are shown in table 5-12.

Table 5-12: Allocation of hyponatraemia diagnosis at time points after baseline

Day of testing	Participants with plasma sodium <135 mmol/L and urinary output measurement at time point	Urinary output criteria (polyuria is defined as urine output \geq 30mls/Kg in 24 hours)	
		CSW	SIADH
Day 3	74	73 (98.6%)	1 (1.4%)
Day 7	87	84 (96.6%)	2 (2.3%)
Day 14	67	67 (100%)	0 (0%)
Day 21	61	59 (96.7%)	2 (3.3%)
Day 28	23	23 (100%)	0 (0%)
Day of testing	Participants with plasma sodium <135 mmol/L and IVC ultrasound measurement at time point	IVC Ultrasound criteria for hypo- and euvolaemia	
		CSW	SIADH
Day 3	20	7 (35.0%)	12 (60%)
Day 7	26	9 (34.6%)	13 (50.0%)
Day 14	17	7 (41.1%)	7 (41.1%)
Day 21	16	7 (43.8%)	8 (50.0%)
Day 28	3	2 (66.7%)	1 (33.3%)
Day of testing	Participants with plasma sodium <135 mmol/L and IVC ultrasound measurement at time point	Urinary sodium criteria for hypo- and euvolaemia (CSW urinary sodium is defined as \geq 100mmol/L)	
		CSW	SIADH
Day 3	54	29 (53.7%)	25 (46.3%)

Day 7	56	31 (55.4%)	25 (44.6%)
Day 14	42	26 (61.9%)	16 (38.1%)
Day 21	32	20 (62.5%)	12 (37.5%)
Day 28	13	7 (53.8%)	6 (46.2%)

Where the sum of CSW and SIADH participants does not match the number of participants with plasma sodium and urinary output, or with plasma sodium and ultrasound (whichever is the corresponding total column), the remaining cases (making up the sum total) were labelled as 'Unknown' by the criteria (see above methods). For IVC ultrasound, 'day 28' scans were performed at day 30 (+/- 1 day) and are here associated with plasma sodium values at day 28 +/- 1 day. CSW=cerebral salt wasting. IVC=inferior vena cava. SIADH=syndrome of inappropriate anti-diuretic hormone secretion.

CSW accounted for nearly all diagnoses of hyponatraemia when using urinary output criteria. Using IVC ultrasound criteria, participants were allocated to CSW and SIADH in nearly equal measure. Using urinary sodium criteria, participants were predominantly allocated to CSW and SIADH.

Allocation of hyponatraemia diagnosis at time points after baseline, using urinary output, IVC ultrasound, and urinary sodium, was then performed stratified by severity of hyponatraemia at that time point (table 5-13). CSW did not appear a more frequent diagnosis with increased severity of hyponatraemia; however, CSW was more frequent at later time points independent of hyponatraemia severity (table 5-12). Interestingly cases of SIADH were proportionally more frequent at worsening hyponatraemia severity (consistent with positive correlation between plasma sodium and urinary sodium in profound hyponatraemia vs. negative correlation between these parameters with mild or moderate hyponatraemia; albeit without statistical significance). A reduction in urinary sodium when plasma sodium is reduced (i.e. positive correlation) may reflect depletion of urinary sodium in profound hyponatraemia.

Table 5-13: Allocation of hyponatraemia diagnosis at time points after baseline, by severity of hyponatraemia at that time point

Day of testing	Severity	Participants with plasma sodium <135 mmol/L and urinary output measurement at time point	Urine output criteria		Participants with plasma sodium <135 mmol/L and IVC ultrasound measurement at time point	IVC ultrasound criteria		Participants with plasma sodium <135 mmol/L and urinary sodium measurement at time point	Urinary sodium criteria	
		N	CSW	SIADH	N	CSW	SIADH		CSW	SIADH
Day 3	Mild	11	11 (100%)	0 (0%)	2	2 (100%)	0 (0%)	22	13 (59.1%)	9 (40.9%)
Day 3	Moderate	31	31 (100%)	0 (0%)	5	3 (60.0%)	2 (40.0%)	17	12 (70.6%)	5 (29.4%)
Day 3	Profound	17	16 (94.1%)	1 (5.9%)	9	3 (33.3%)	5 (66.6%)	15	4 (26.7%)	11 (73.3%)
Day 7	Mild	40	37 (92.5%)	0 (0%)	3	3 (100%)	0 (0%)	24	13 (54.2%)	11 (45.8%)
Day 7	Moderate	26	26 (100%)	0 (0%)	14	5 (35.7%)	4 (28.6%)	16	13 (81.3%)	3 (18.8%)
Day 7	Profound	21	21 (100%)	0 (0%)	4	0 (0%)	1 (25.0%)	16	5 (31.3%)	11 (68.8%)

Day 14	Mild	32	32 (100%)	0 (0%)	3	0 (0%)	0 (0%)	18	13 (72.2%)	5 (27.8%)
Day 14	Moderate	20	20 (100%)	0 (0%)	7	3 (42.9%)	0 (0%)	15	12 (80.0%)	3 (20.0%)
Day 14	Profound	15	15 (100%)	0 (0%)	3	1 (33.3%)	0 (0%)	9	1 (11.1%)	8 (88.9%)
Day 21	Mild	32	31 (96.9%)	1 (3.1%)	3	0 (0%)	1 (33.3%)	18	13 (72.2%)	5 (27.8%)
Day 21	Moderate	20	19 (95.0%)	1 (5.0%)	5	3 (60.0%)	1 (20.0%)	9	4 (44.4%)	5 (55.6%)
Day 21	Profound	9	9 (100%)	0 (0%)	4	2 (50.0%)	1 (25.0%)	5	3 (60.0%)	2 (40.0%)
Day 28	Mild	7	7 (100%)	0 (0%)	1	1 (100%)	0 (0%)	3	3 (100%)	0 (0%)
Day 28	Moderate	7	7 (100%)	0 (0%)	1	1 (100%)	0 (0%)	3	2 (66.6%)	1 (33.3%)
Day 28	Profound	9	9 (100%)	0 (0%)	2	1 (50.0%)	0 (0%)	7	2 (28.6%)	5 (71.4%)

‘Severity’ is defined as severity of hyponatraemia, as per study methods. Where the sum of CSW and SIADH participants does not match the number of participants with plasma sodium and urinary output, or match plasma sodium and ultrasound (whichever is the corresponding total column), the remaining cases (making up the sum total) were labelled as ‘Unknown’ by the criteria (see above methods). For IVC ultrasound, ‘day 28’ scans were performed at day 30 (+/- 1 day) and are here associated with plasma sodium values at day 28 +/- 1 day. CSW=cerebral salt wasting. IVC=inferior vena cava. N=number of participants. SIADH=syndrome of inappropriate anti-diuretic hormone secretion.

5.3.8 Sodium parameters during the first 30 days of anti-tuberculous chemotherapy

The number of plasma and urinary sodium, serum and urinary osmolality, and fluid balance parameters, measured at each time point are shown in table 5-14. A higher number of tests were performed at earlier time points largely due to deaths of participants given the high mortality associated with TBM, or discharge from the study site.

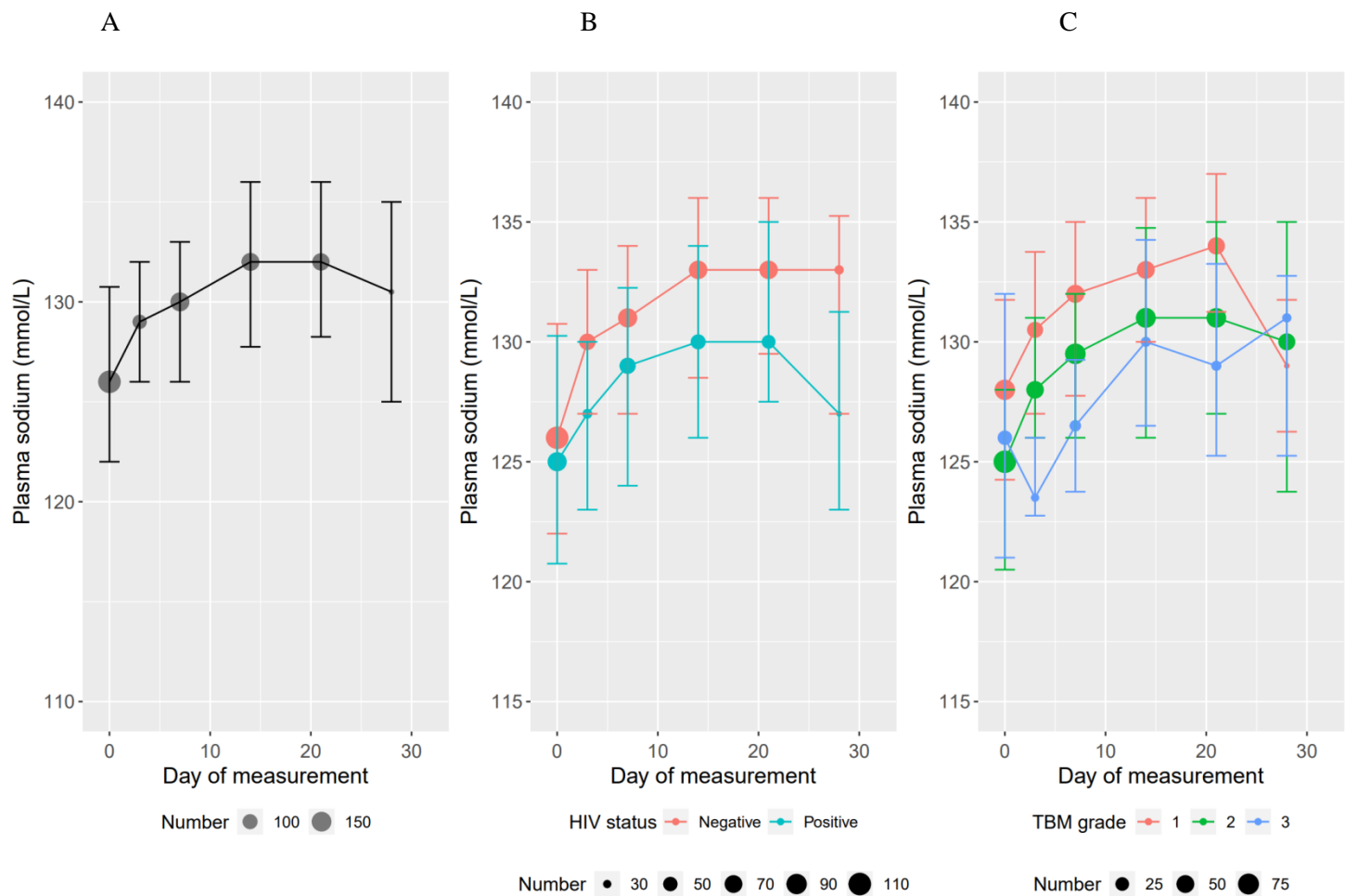
Table 5-14: The number of tests at each time point

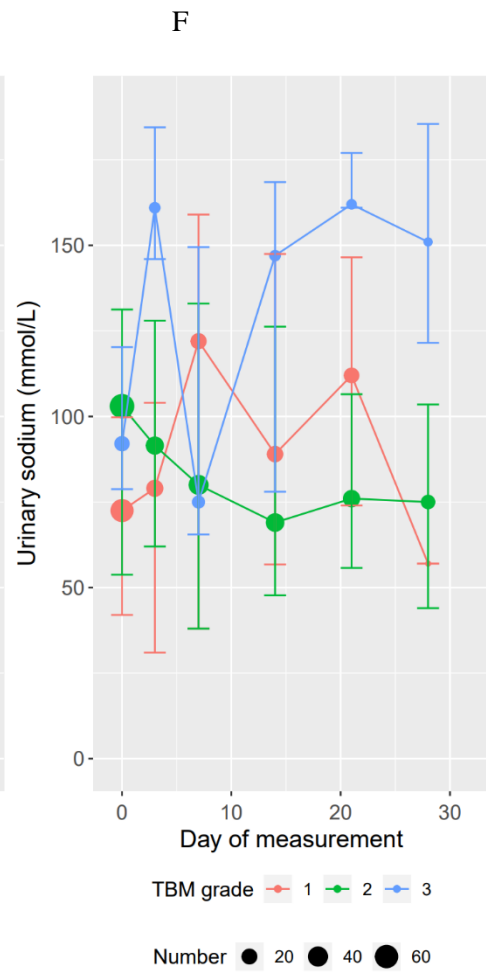
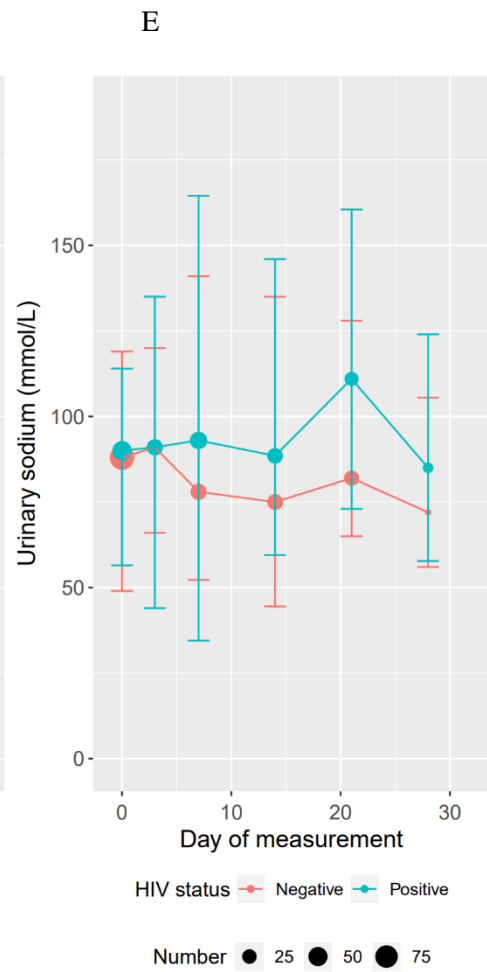
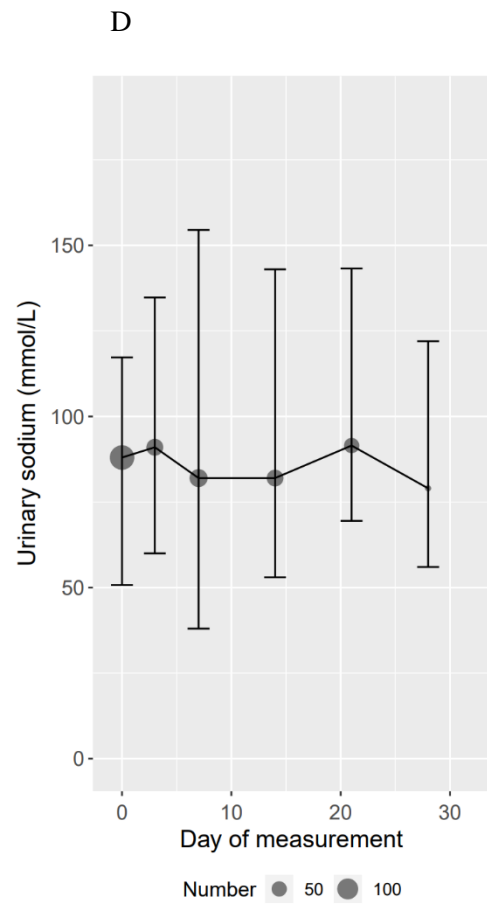
	Day 0 tests	Day 3 tests	Day 7 tests	Day 14 tests	Day 21 tests	Day 28 tests
Plasma sodium (mmol/L)	190	94	140	128	122	60
Urinary sodium (mmol/L)	144	62	71	61	50	19
Serum osmolality (mOsm/Kg)	158	127	140	140	125	53
Urinary osmolality (mOsm/Kg)	152	126	138	133	126	53
24-hour fluid balance (mls)	166	138	137	135	127	66
24-hour fluid output (mls)	166	136	136	134	126	6

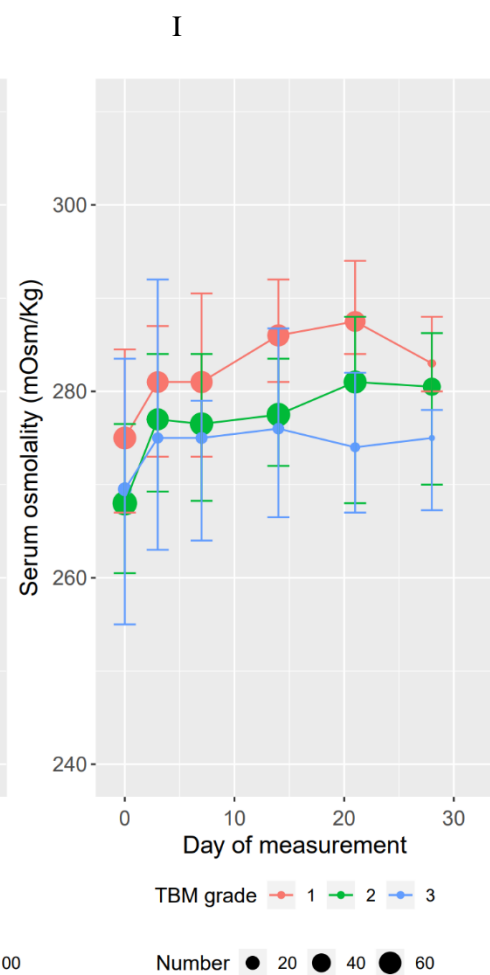
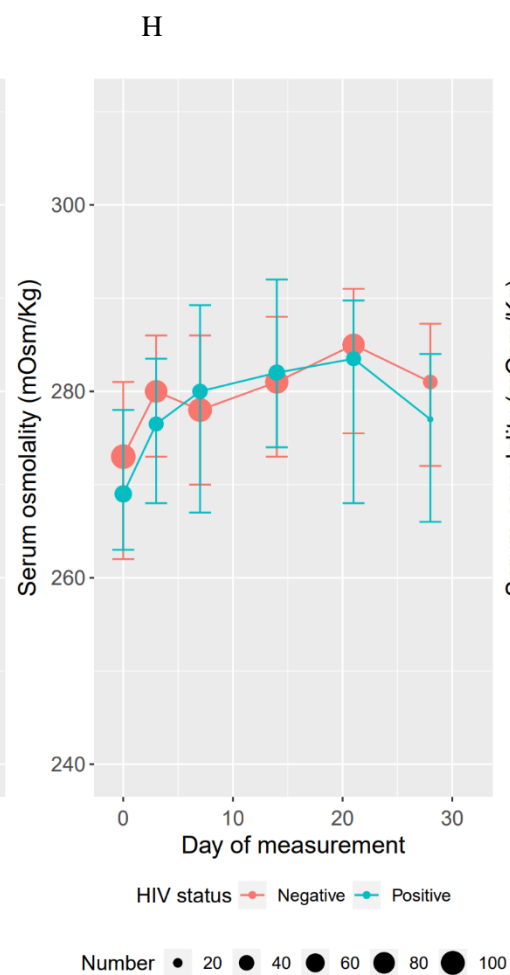
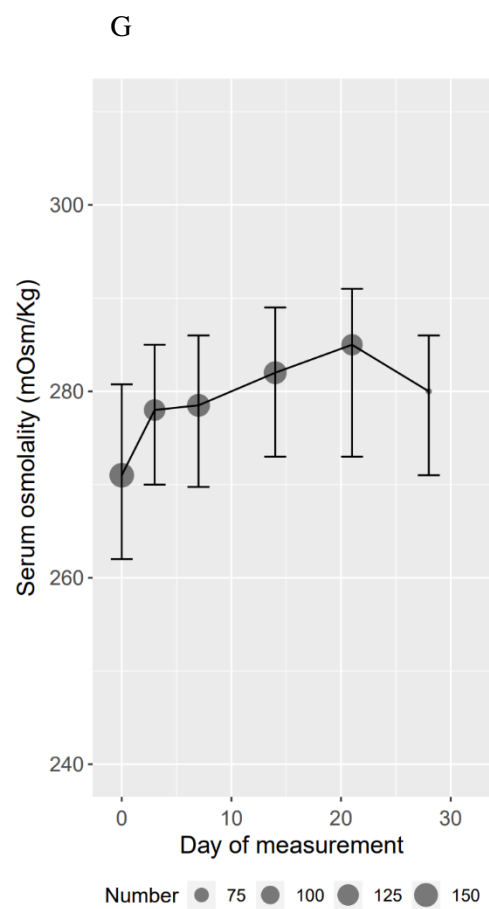
Each number represents the number of tests performed. Values of tests are not displayed in this table.

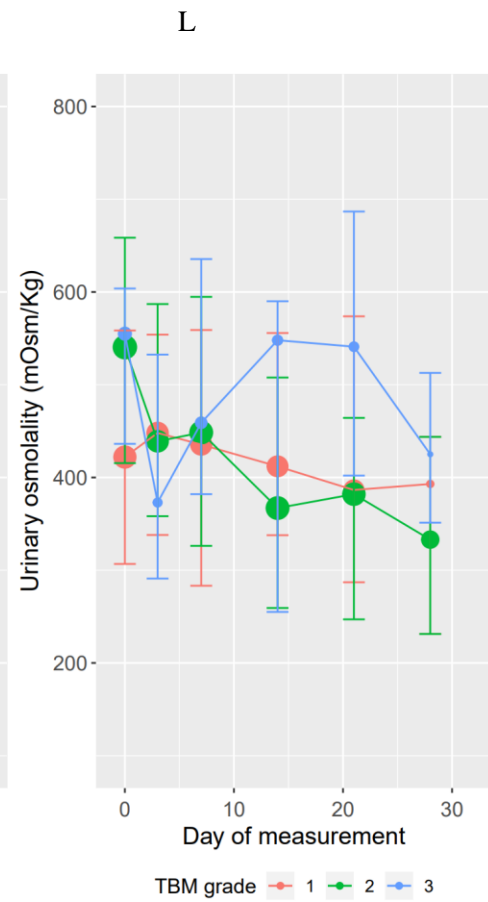
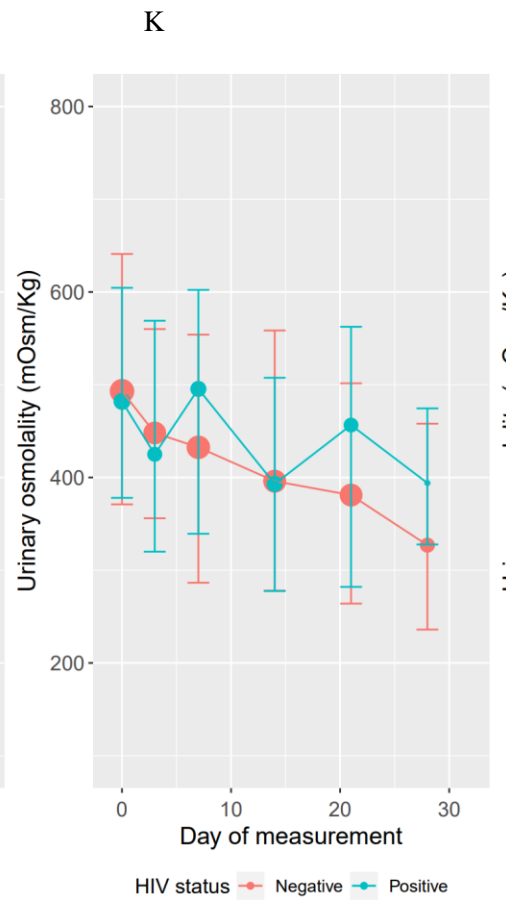
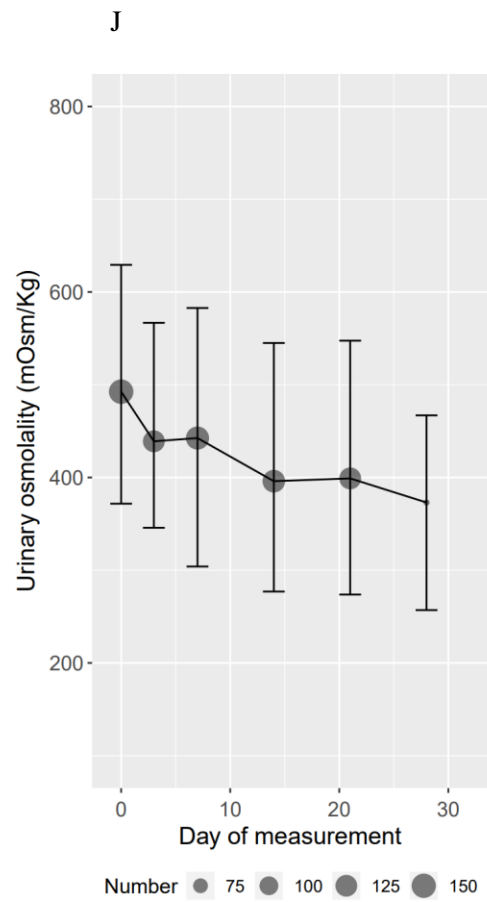
Median sodium parameters with IQR are shown in figure 5-9, for all participants, and stratified by HIV co-infection status, and by MRC TBM severity grade. These data illustrate a trend of lower plasma sodium, higher urinary sodium, higher urinary osmolality, and higher urinary output in HIV co-infected participants vs. HIV uninfected participants. This same trend was seen in more severe TBM disease (higher MRC TBM severity grade), in addition to lower serum osmolality.

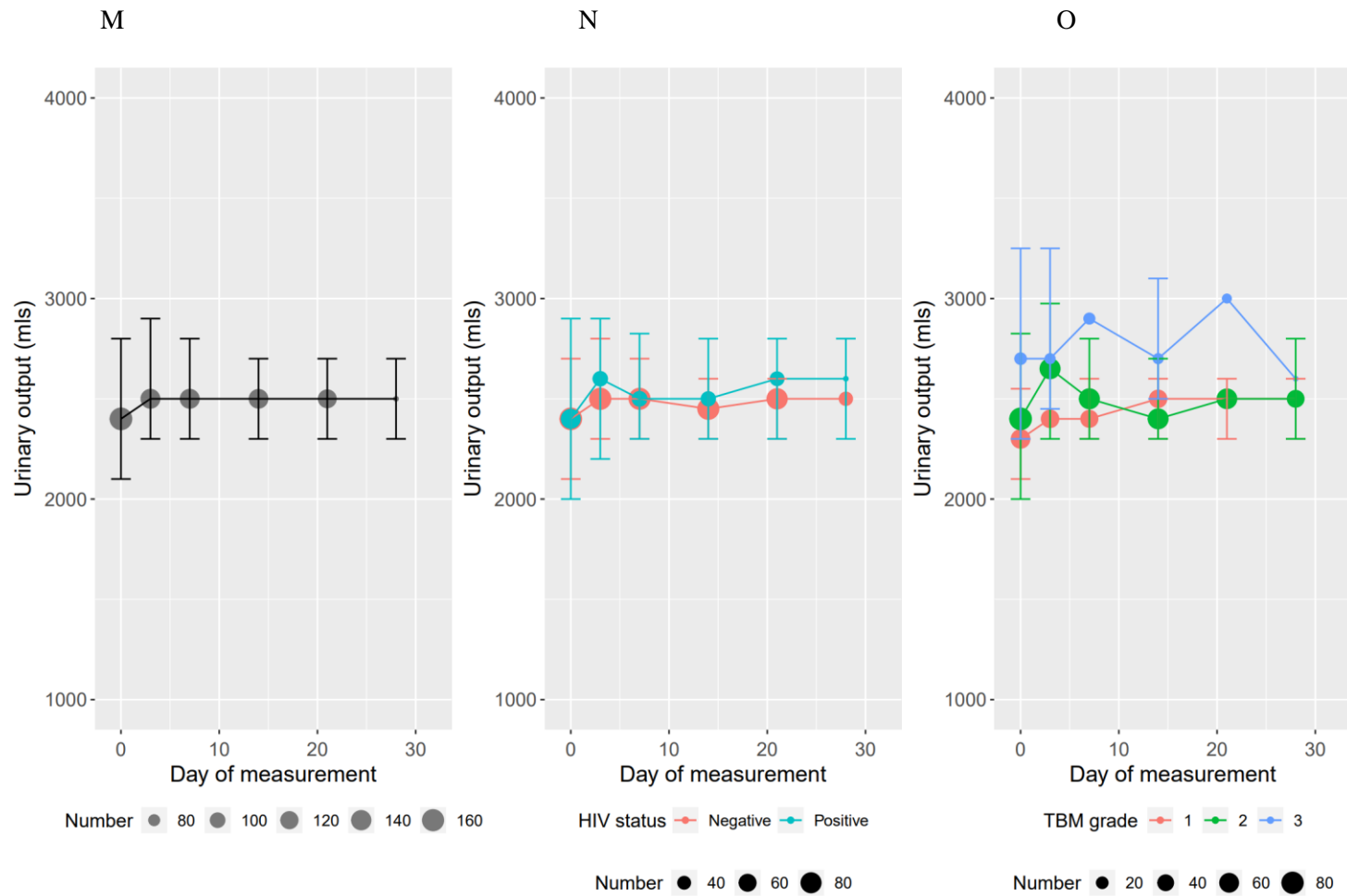
Figure 5-9: Sodium parameters shown in total, and stratified by HIV co-infection and TBM severity grade







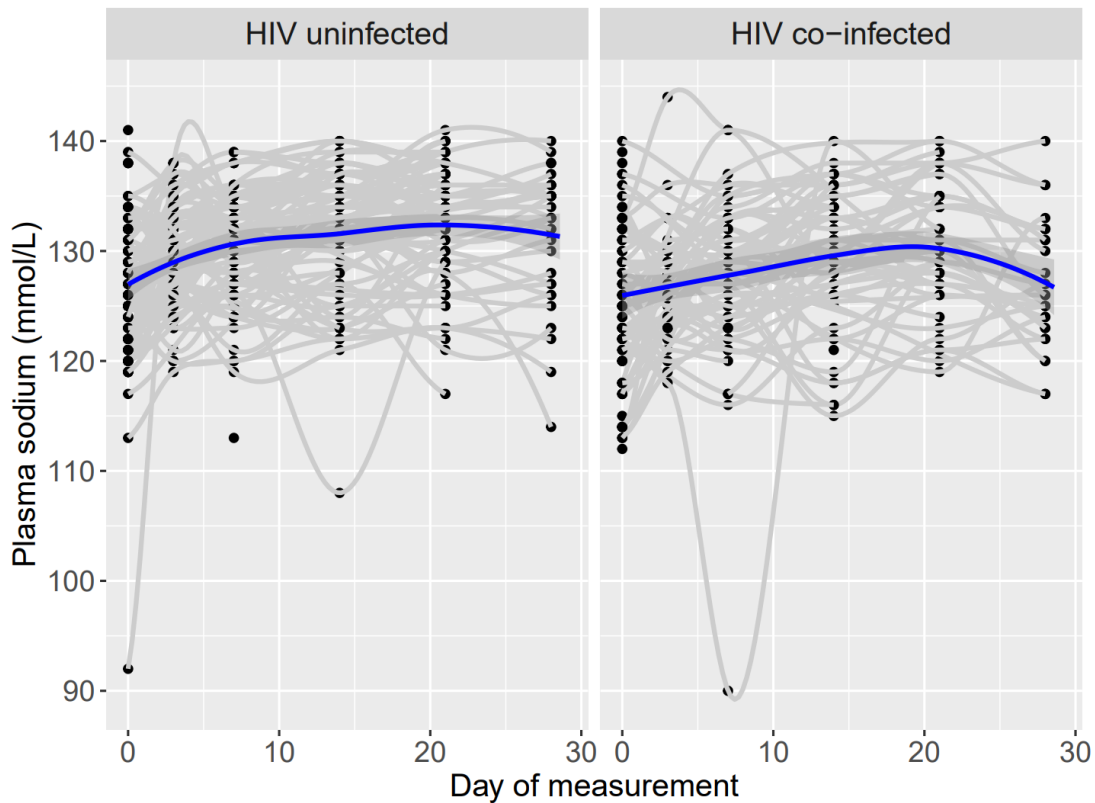




Data are shown for plasma sodium (panels A-C), urinary sodium (panels D-F), serum osmolality (panels G-I), urinary osmolality (panels J-L), and urinary output (panels M-O). Median values are shown for each parameter at each time point. The size of each median value point represents the number of samples contributing to the median value at this point. Median values are connected by a central line. The difference between upper and lower error bars represents the interquartile range.

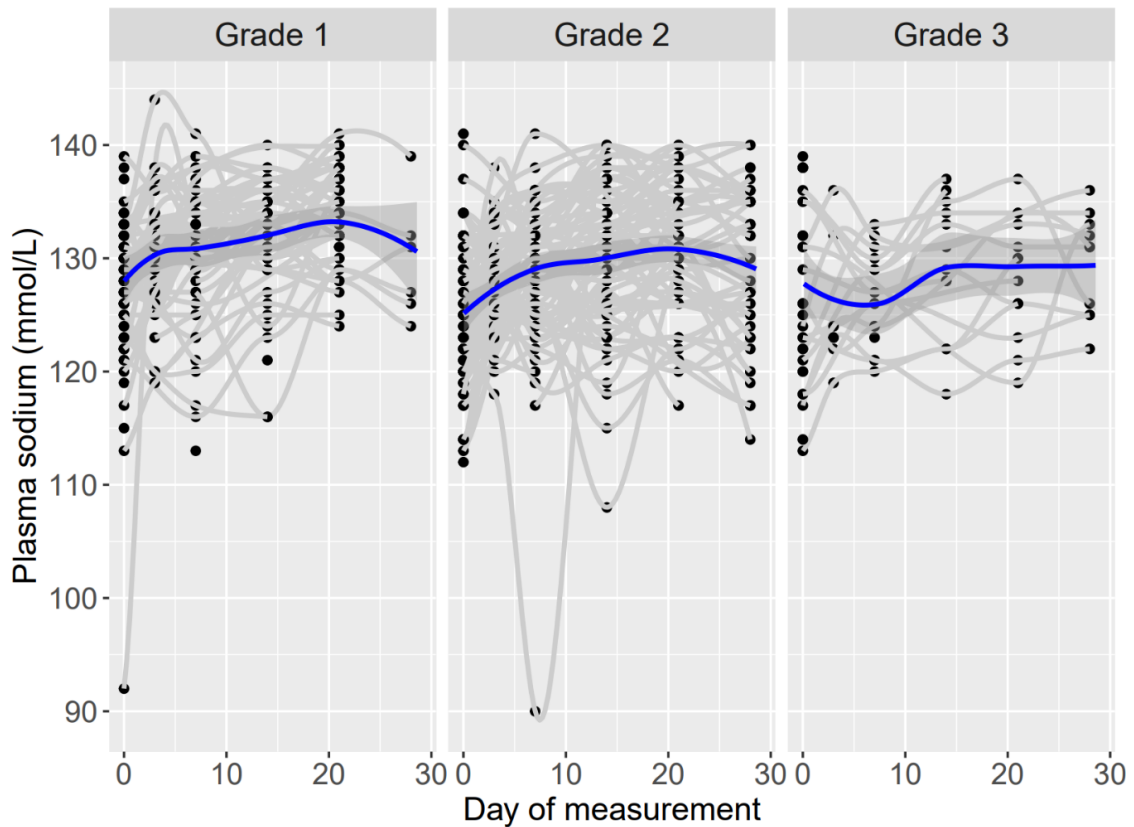
Individual participant plasma sodium data is shown by HIV status (figure 5-10) and by MRC TBM severity grade (figure 5-11).

Figure 5-10: Individual participant plasma sodium over the first 30 days of anti-TB chemotherapy, by HIV co-infection status



Individual points represent individual participants' measurements of plasma sodium. A central blue line of best fit is surrounded by grey shading defining the 95% confidence interval. Light grey lines represent individual participant plasma sodium trends.

Figure 5-11: Individual participant plasma sodium over the first 30 days of anti-TB chemotherapy, by TBM severity grade



Individual points represent individual participants' measurements of plasma sodium. A central blue line of best fit is surrounded by grey shading defining the 95% confidence interval. Light grey lines represent individual participant plasma sodium trends. Grade represents Medical Research Council tuberculous meningitis severity grade.

5.3.9 The use of hypertonic saline therapy

Electronic data describing hypertonic saline 3%, saline 0.9%, and furosemide 40mg, including fluid volume, and time and date of administration, were available for 205/208 (98.6%) participants.

Mannitol use at HTD is exceptionally unusual; therefore, data for this fluid was not collected.

There were 19 doses of furosemide used in 6 participants (totaling 420mg). In total 17004 sodium containing therapies (3% or 0.9% sodium chloride) were used in 169/205 (82.4%) participants. In total 1,194 litres of hypertonic saline 3% were used in 142/205 (69.3%) participants (mean ~8.4 litres per participant).

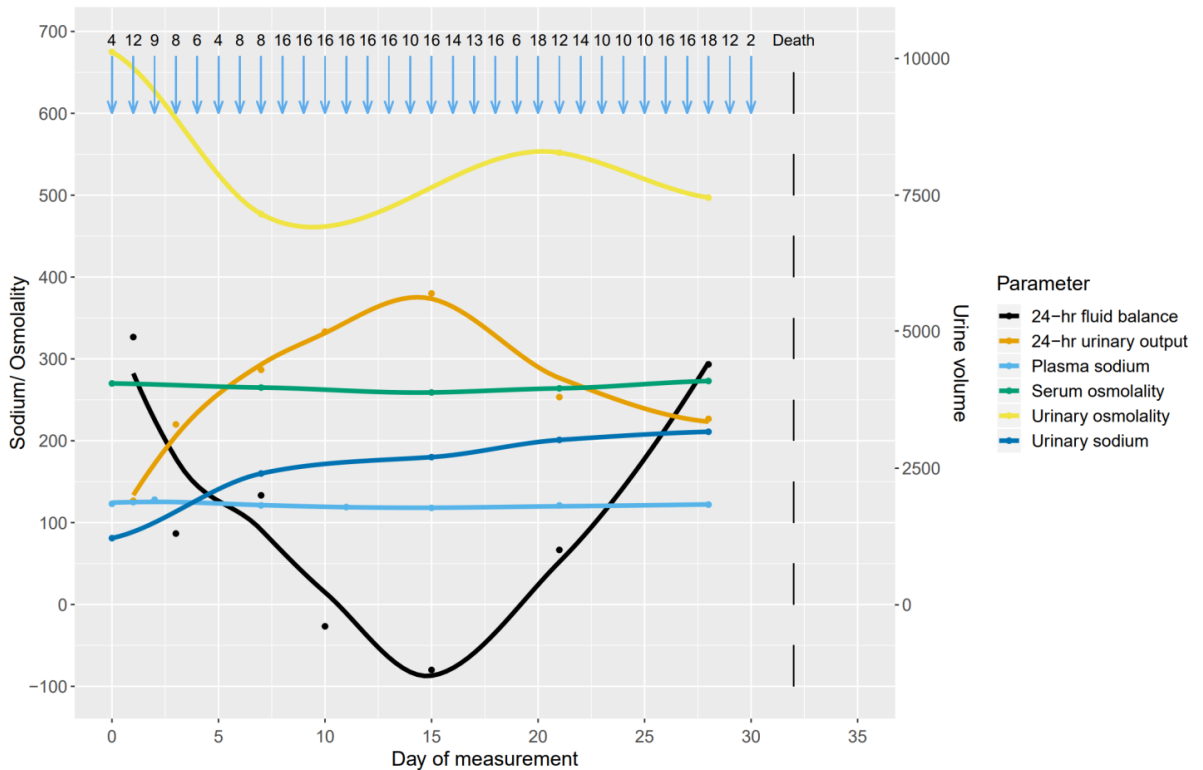
Of these 1,194 litres of hypertonic saline 3%, 777 litres (65.0%) were administered to 70 HIV co-infected participants, and 418 litres (35.0%) were administered to 72 HIV uninfected participants. By MRC TBM severity grade, 150 litres (12.6%) were administered to 40 grade 1 participants, 761 litres (63.7%) were administered to 80 grade 2 participants, and 283 litres (23.7%) were administered to 22 grade 3 participants. During the first 30 days of from study randomisation, 132 participants received 673 litres of hypertonic saline 3% (mean ~5.1 litres per participant).

Use of hypertonic saline 3% vials per day was greatest in the most severely ill participants. However total use was lower in the most severely ill participants due to earlier death. In a treatment subgroup of participants with hyponatraemia at baseline (n=176), hypertonic saline 3% administration was stratified by baseline hyponatraemia severity; mild hyponatraemia: 31 litres, 17/42 (40.5%) participants; moderate hyponatraemia: 57 litres, 37/59 (62.7%) participants; profound hyponatraemia: 172 litres, 65/75 (86.7%) participants. The use of hypertonic saline 3% in the treatment plans of two individual participants is described in figures 5-12 and 5-13.

5.3.10 Assessing individual participants

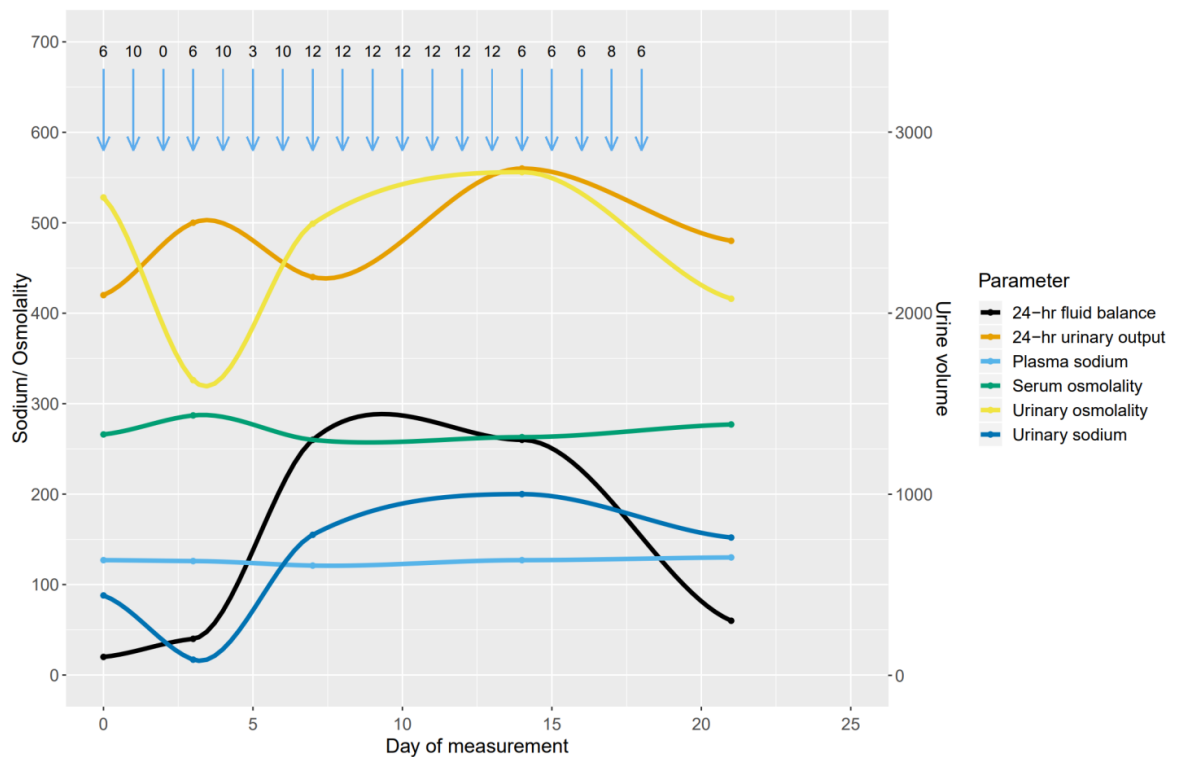
Individual participant analysis was performed for two contrasting participants (figures 5-12 and 5-13), with measured variables, outcome, and use of hypertonic saline 3%. Participants were selected to reflect severe disease (baseline grade 3, HIV co-infected), and non-severe disease (baseline grade 1, HIV uninfected), respectively, with the two participants with the two most complete datasets then selected.

Figure 5-12: Sodium parameters in a grade 3 HIV co-infected participant with TBM



Data are shown for a HIV co-infected participant with grade 3 TBM at baseline. Plasma sodium values were as follows: baseline: 123 mmol/L, day 7: 121 mmol/L, day 15: 118 mmol/L, day 21: 121 mmol/L, day 28: 122 mmol/L. Sodium represents plasma sodium and urine sodium measured in mmol/L. Osmolality represents plasma and urine osmolality measured in mOsm/Kg. Blue arrows indicate the days of hypertonic saline 3% administration, with the black number above each arrow indicating the number of 100mls vials of hypertonic saline 3% received on that day. HIV=human immunodeficiency virus. Hr=hour. Kg=kilogram. TBM=tuberculous meningitis.

Figure 5-13: Sodium parameters in a grade 1 HIV uninfected participant with TBM



Data are shown for a HIV uninfected participant with grade 1 TBM at baseline. Plasma sodium values were as follows: plasma sodium values were as follows: baseline (day 0): 127 mmol/L, day 3: 126 mmol/L, day 7: 121 mmol/L, day 14: 127 mmol/L, day 21: 130 mmol/L. Sodium represents plasma sodium and urine sodium measured in mmol/L. Osmolality represents plasma and urine osmolality measured in mOsm/Kg. Blue arrows indicate the days of hypertonic saline 3% administration, with the black number above each arrow indicating the number of 100mls vials of hypertonic saline 3% received on that day. HIV=human immunodeficiency virus. Hr=hour. Kg=kilogram. TBM=tuberculous meningitis.

In these two illustrations the following can be observed: firstly, more hypertonic saline 3% is received in the grade 3 participant (figure 5-13), who remains grade 3 throughout the illness, with death occurring on day 32. Plasma sodium remains low in this grade 3 participant (122 mmol/L on day 28) but shows partial recovery in the grade 1 participant (figure 5-14). Urinary outputs are at least two times greater in the grade 3 participant (reaching more than 5000mls). During a period of hypertonic saline 3% administration urinary sodium rises in both participants.

5.3.11 Clinical outcome

5.3.11.1 Plasma sodium and clinical outcome

The associations between baseline plasma sodium and clinical outcomes are shown by subgroup in table 5-15.

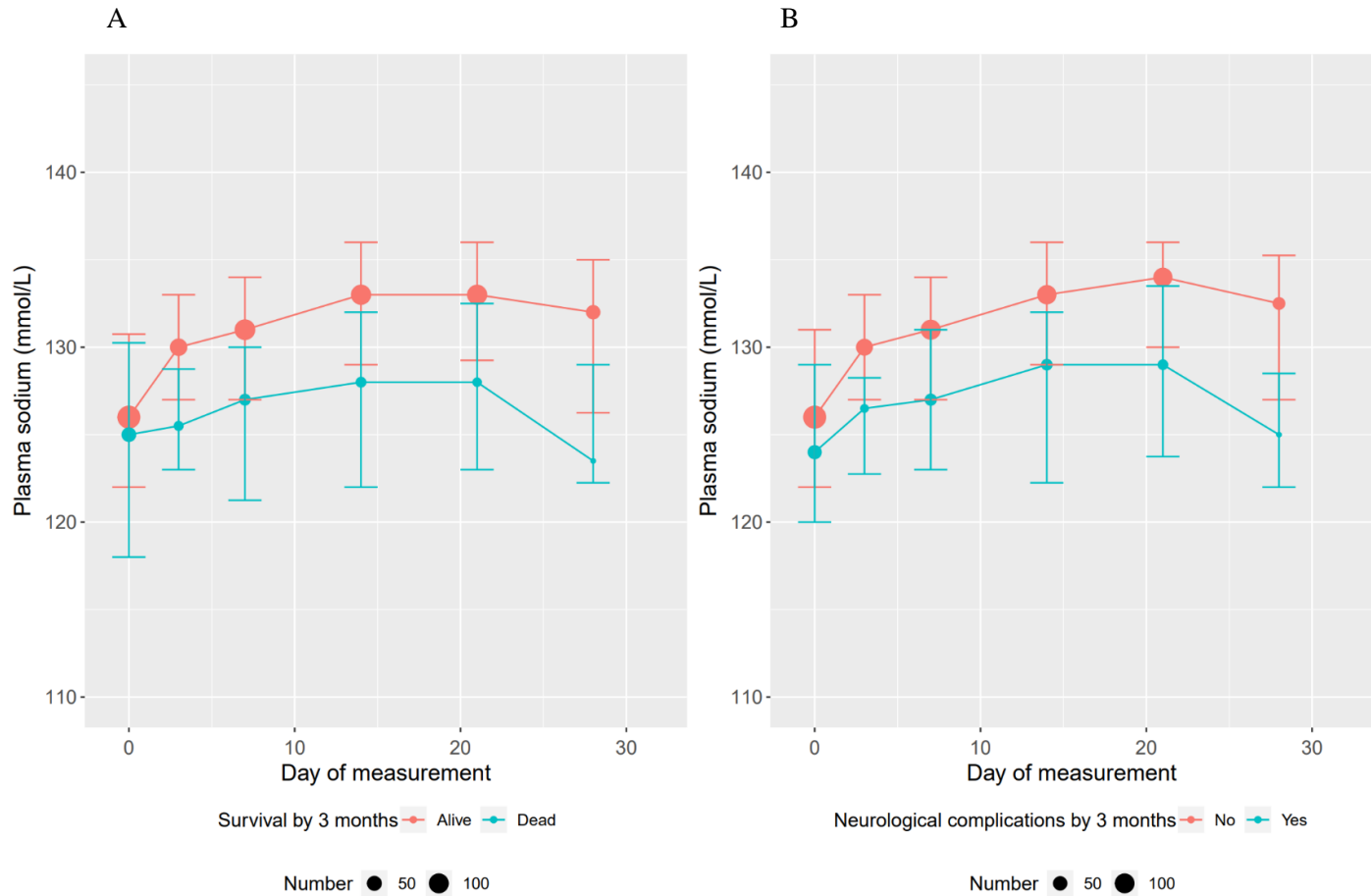
Table 5-15: Baseline plasma sodium and clinical outcomes

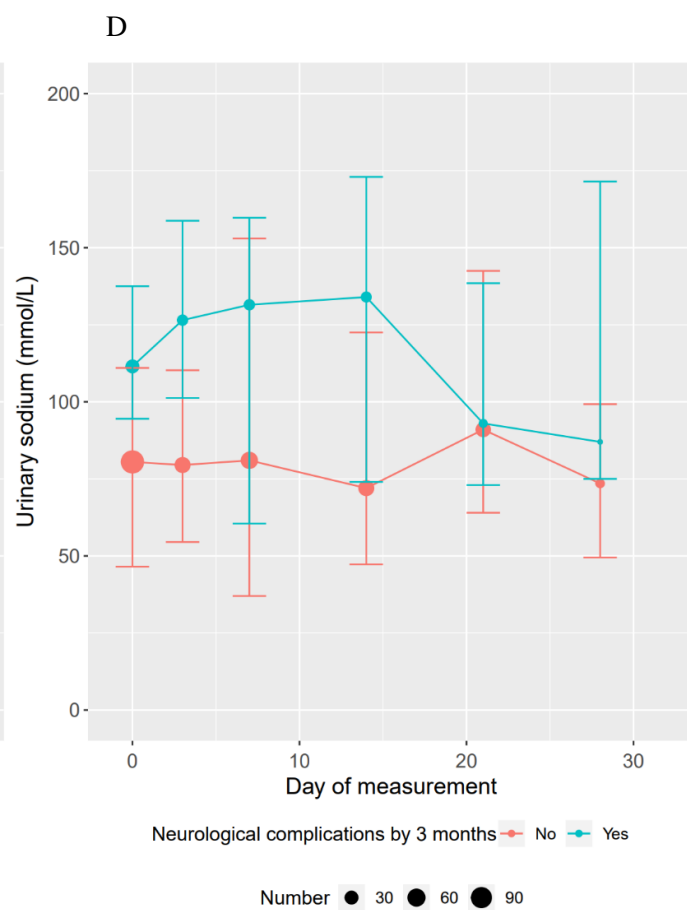
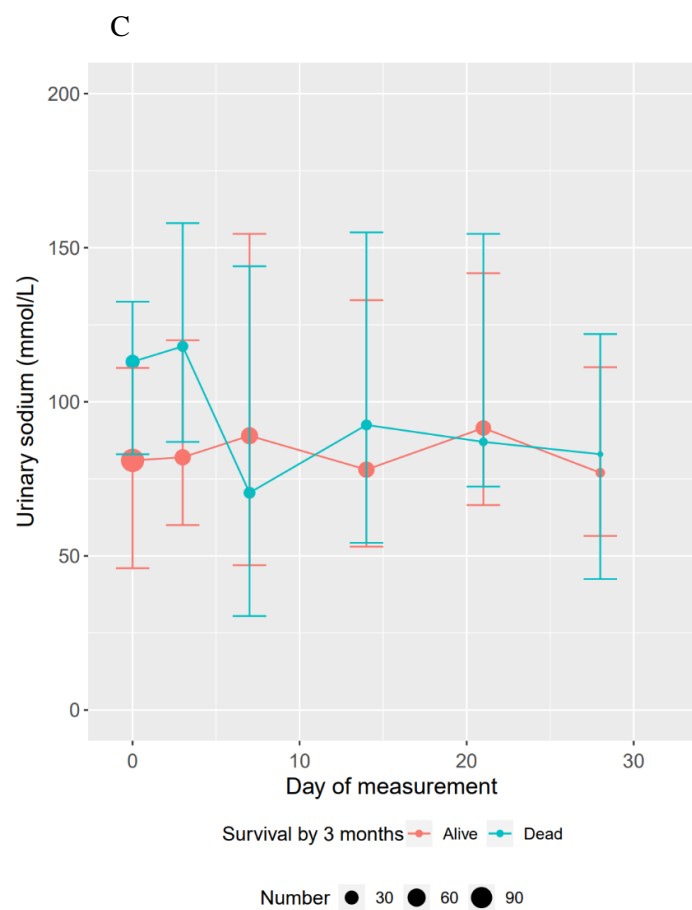
	No. participants	Plasma sodium (mmol/L)	P value
All participants (Median[IQR])	190	126 (125-127)	
Survival by 3 months (Median[IQR])			
- Alive	142	126 (122-131)	0.14
- Dead	48	125 (118-130)	
Neurological complications by 3 months (Median[IQR])			
- No	141	126 (122-131)	0.05
- Yes	49	124 (120-129)	

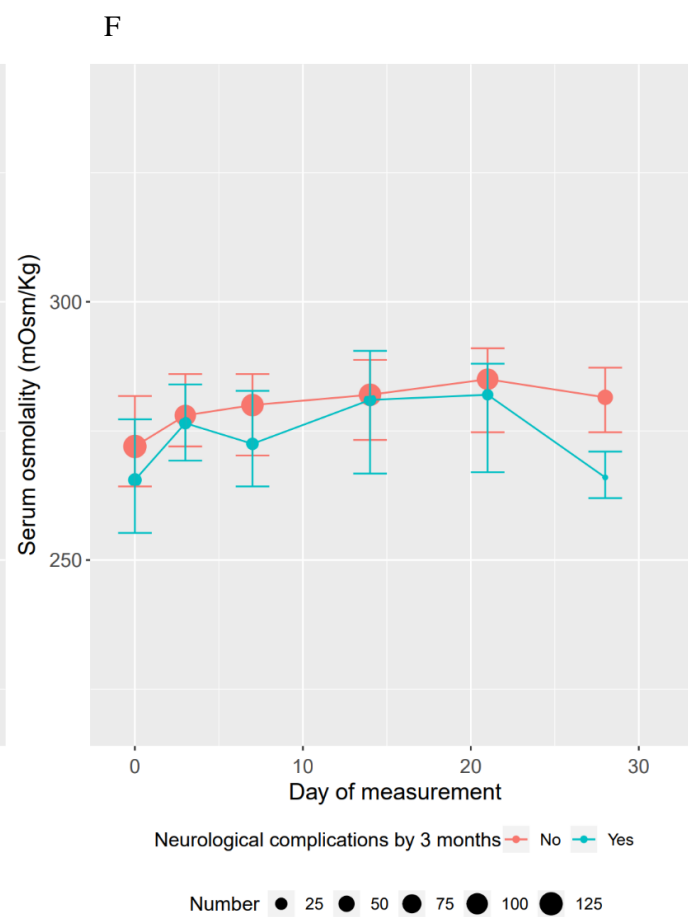
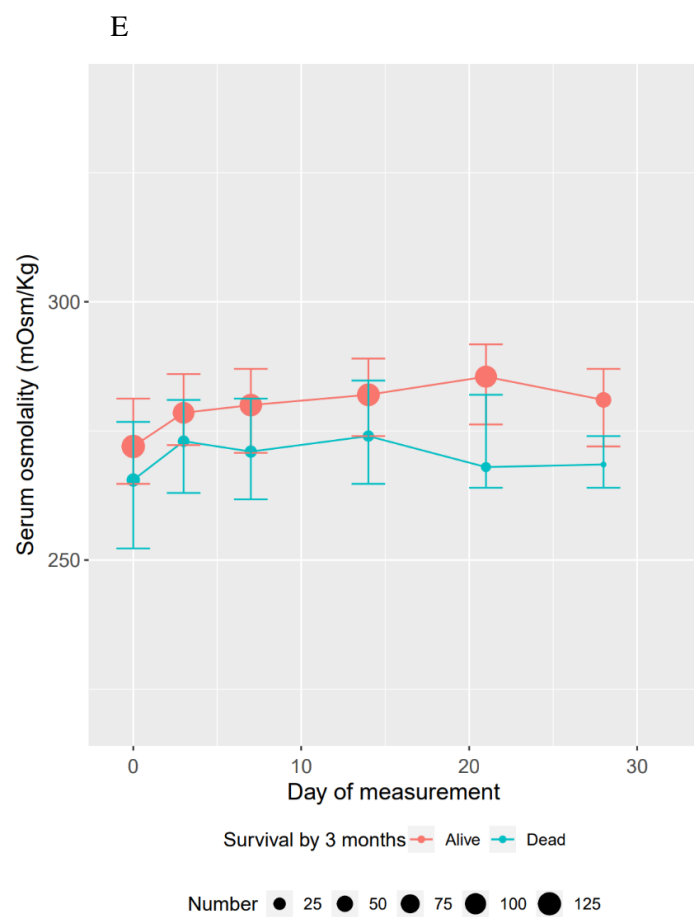
Median plasma sodium value is shown with IQR. P value represents Wilcoxon rank sum test; given plasma sodium values were non-normally distributed. HIV=human immunodeficiency virus. IQR=interquartile range. MRC=Medical Research Council. TBM=tuberculous meningitis.

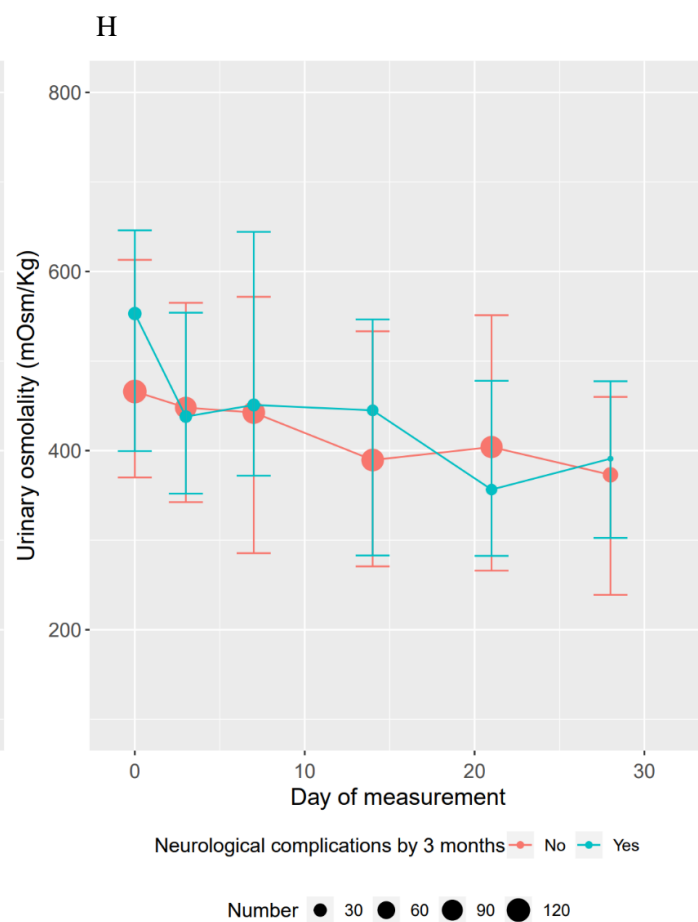
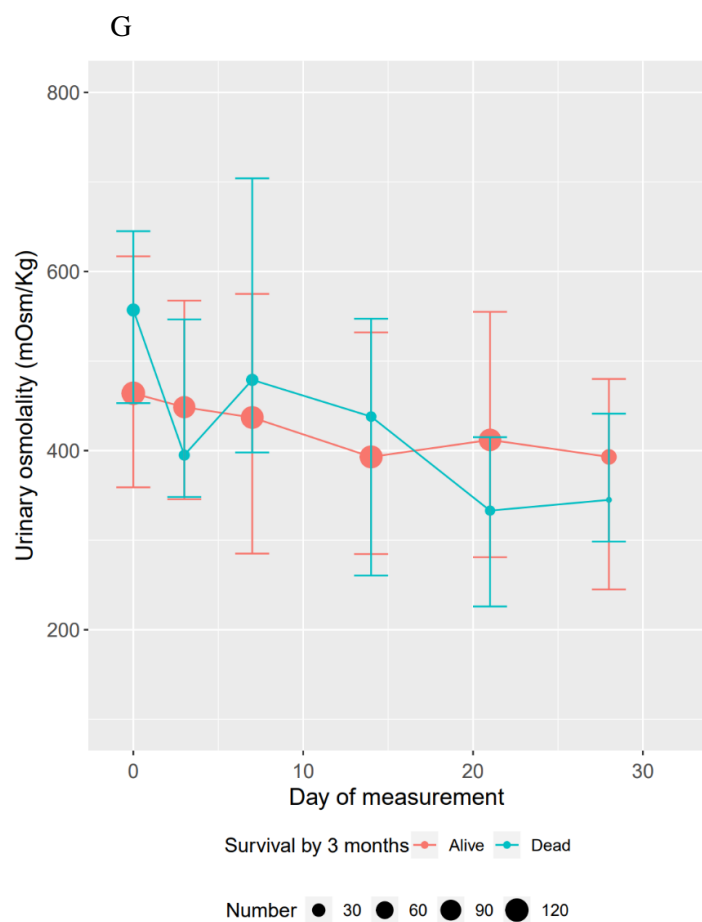
A trend towards a significant association was seen between lower baseline plasma sodium and neurological complication by 3 months ($p=0.05$). In figure 5-14 sodium parameters are shown in total, and stratified by survival by 3 months, and by neurological complications by 3 months. Data show trends of reduced survival by 3 months, and more neurological complications by 3 months, in participants with lower plasma sodium, and participants with higher urinary output.

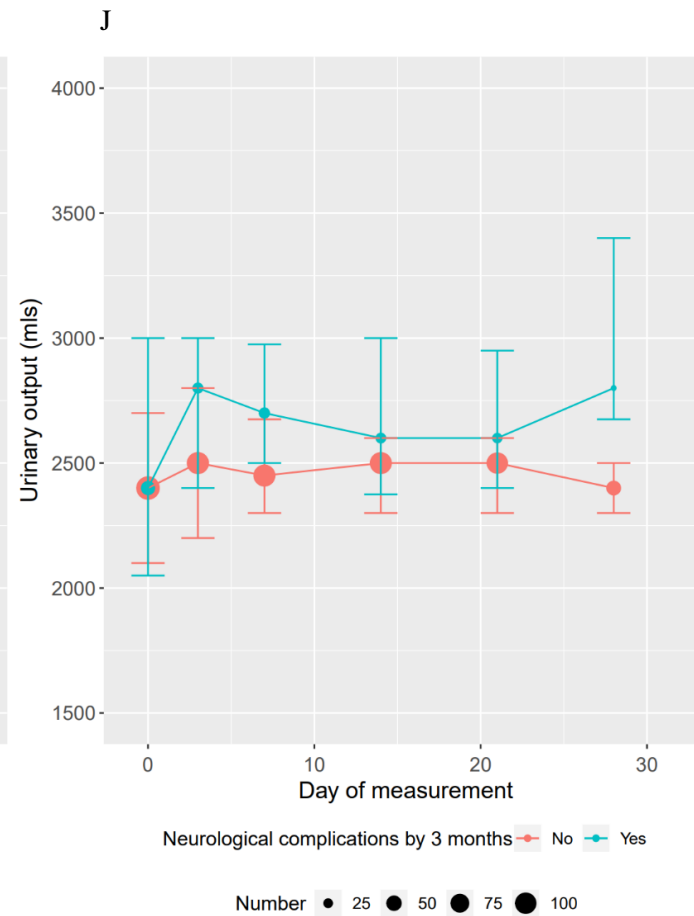
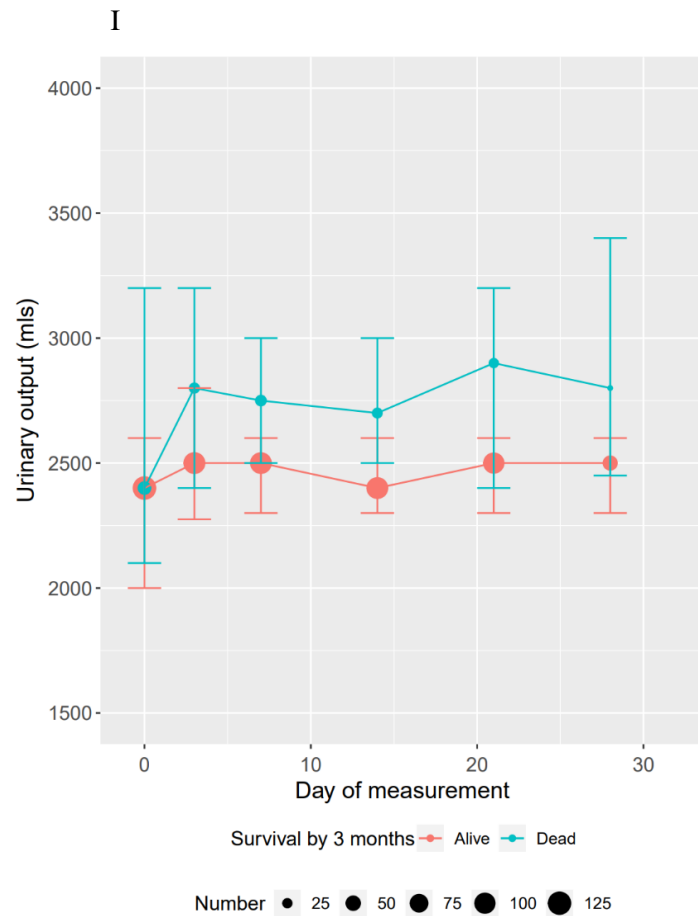
Figure 5-14: Sodium parameters shown in total, and stratified by survival by 3 months, and by neurological complications by 3 months







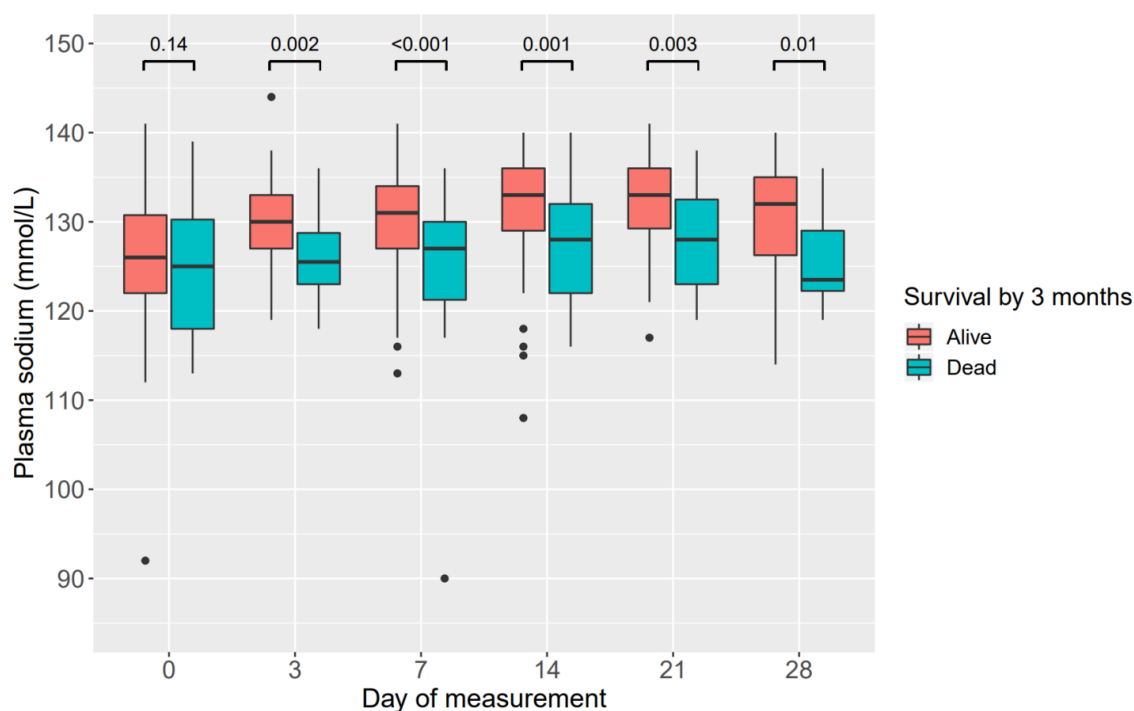




Data are shown for plasma sodium (panels A-B), urinary sodium (panels C-D), serum osmolality (panels E-F), urinary osmolality (panels G-H), and urinary output (panels I-J). Median values are shown for each parameter at each time point. The size of each median value point represents the number of samples contributing to the median value at this point. Median values are connected by a central line. The difference between upper and lower error bars represents the interquartile range.

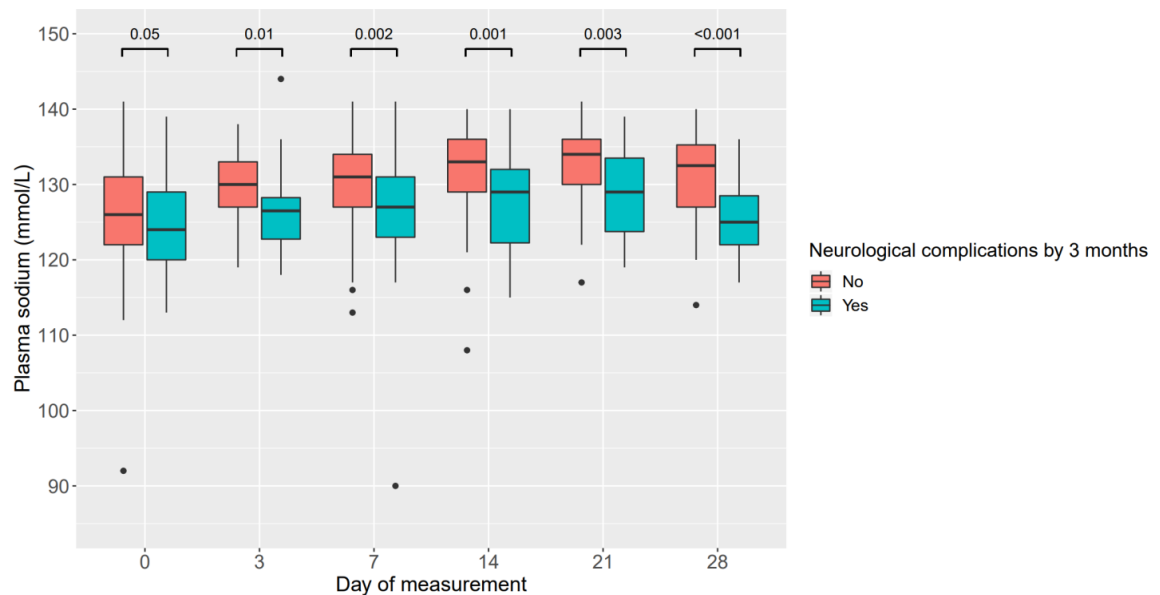
Plasma sodium was significantly lower at all time points after baseline in participants who died by 3 months (figure 5-15) and in participants who experienced neurological complications by 3 months (figure 5-16).

Figure 5-15: Plasma sodium stratified by survival by 3 months



For each individual boxplot, the central horizontal bar represents the median value. The box contains data between 3rd quartile (upper end of box) and 1st quartile (lower end of box). Vertical lines above and below each box extend to the most extreme data point that is within 1.5x the vertical height of the box. Dots represent individual data points. 'Alive' represents participants alive by 3 months. 'Dead' represents participants who died by 3 months. P values represent statistical comparison of groups by the Wilcoxon rank sum test, given data across all time points included non-normally distributed data.

Figure 5-16: Plasma sodium stratified by neurological complications by 3 months

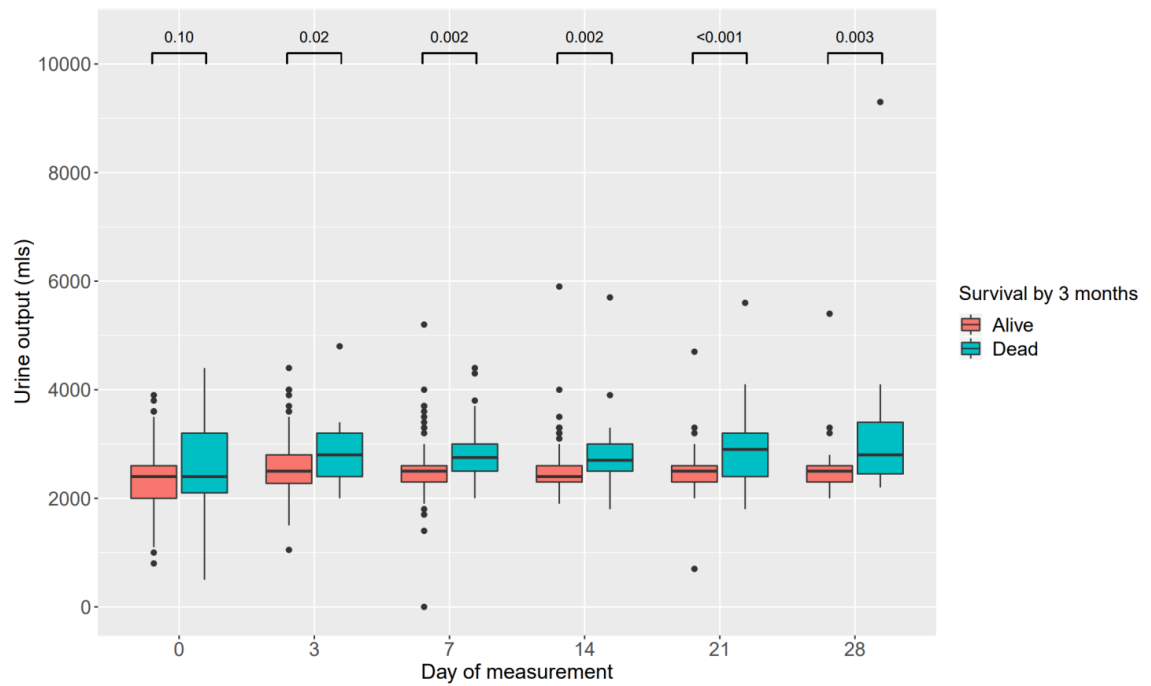


For each individual boxplot, the central horizontal bar represents the median value. The box contains data between 3rd quartile (upper end of box) and 1st quartile (lower end of box). Vertical lines above and below each box extend to the most extreme data point that is within 1.5x the vertical height of the box. Dots represent individual data points. 'No' represents participants who did not experience neurological complications by 3 months. 'Yes' represents participants who experienced neurological complications by 3 months.

5.3.11.2 Urinary output and clinical outcome

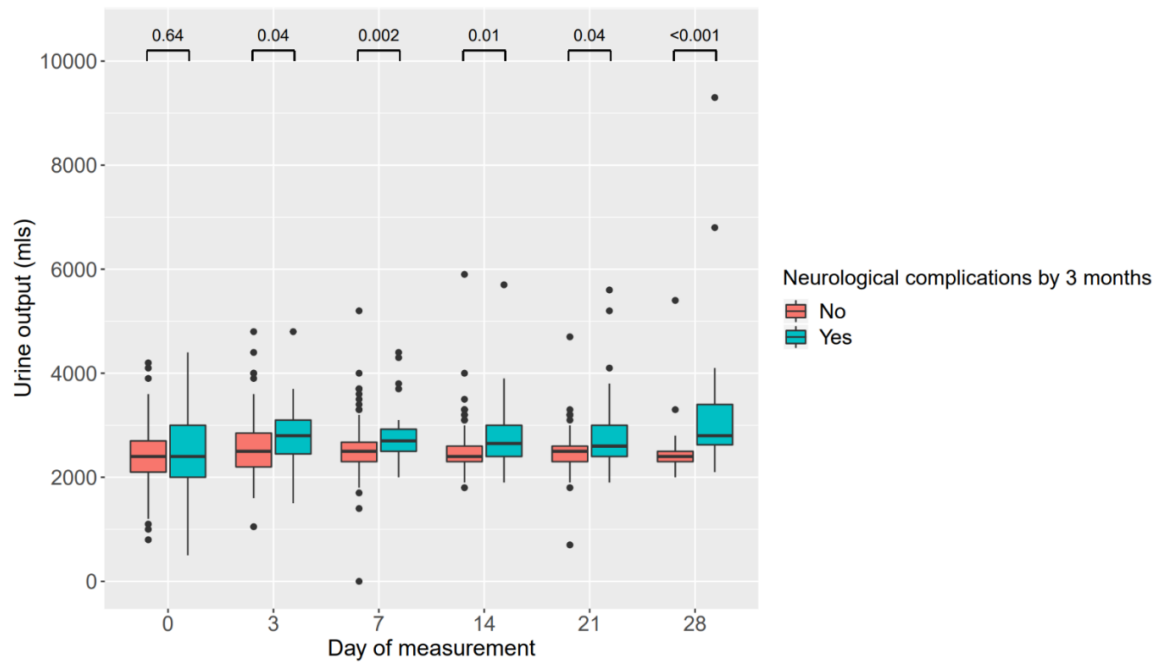
24-hour urinary output was significantly higher at all time points after baseline in participants who died by 3 months (figure 5-17), and in participants who experienced neurological complications by 3 months (figure 5-18). An increased urinary volume favours a diagnosis of CSW over SIADH, in those participants with poor outcomes. However, in data assessing hyponatraemia causality (table 5-13 and 5-14), intravascular assessment (by IVC ultrasound) did not support intravascular depletion in most participants (widespread intravascular depletion would have supported a conclusion of CSW as the common aetiology of hyponatraemia in TBM).

Figure 5-17: Urinary output stratified by survival by 3 months



For each individual boxplot, the central horizontal bar represents the median value. The box contains data between 3rd quartile (upper end of box) and 1st quartile (lower end of box). Vertical lines above and below each box extend to the most extreme data point that is within 1.5x the vertical height of the box. Dots represent individual data points. P values represent statistical comparison of groups by the Wilcoxon rank sum test.

Figure 5-18: Urinary output stratified by neurological complications by 3 months



For each individual boxplot, the central horizontal bar represents the median value. The box contains data between 3rd quartile (upper end of box) and 1st quartile (lower end of box). Vertical lines above and below each box extend to the most extreme data point that is within 1.5x the vertical height of the box. Dots represent individual data points. P values represent statistical comparison of groups by the Wilcoxon rank sum test.

5.3.11.3 IVC ultrasound and clinical outcome

In a restrictive analysis of participants undergoing IVC ultrasound, hypovolaemia assessed by IVC ultrasound is shown stratified by survival by 3 months, and by neurological complications by 3 months, in tables 5-16 and 5-17 respectively. Hypovolaemia assessed by IVC ultrasound was not predictive of death by 3 months, or of neurological complications by 3 months, in this population.

Table 5-16: Hypovolaemia by IVC ultrasound stratified by survival by 3 months

Day of assessment	Number of participants	Hypovolaemia by IVC ultrasound		Number of participants	No hypovolaemia by IVC ultrasound	
		Dead (%)	Alive (%)		Dead (%)	Alive (%)
0	18	5 (27.8%)	13 (72.2%)	38	9 (23.7%)	29 (76.3%)
3	13	1 (7.7%)	12 (92.3%)	24	6 (25.0%)	18 (75.0%)
7	13	2 (15.4%)	11 (84.6%)	17	5 (29.4%)	12 (70.6%)
14	15	3 (20.0%)	12 (80.0%)	15	6 (40.0%)	9 (60.0%)
21	12	3 (25.0%)	9 (75.0%)	23	9 (39.1%)	14 (60.9%)
30 *	5	2 (40.0%)	3 (60.0%)	3	2 (66.6%)	1 (33.3%)

* For IVC ultrasound, 'day 28' scans were performed at day 30 (+/- 1 day). For consistency throughout this results chapter they are again referred to as 'day 28' here. IVC=inferior vena cava.

Table 5-17: Hypovolaemia by IVC ultrasound stratified by neurological complications by 3 months

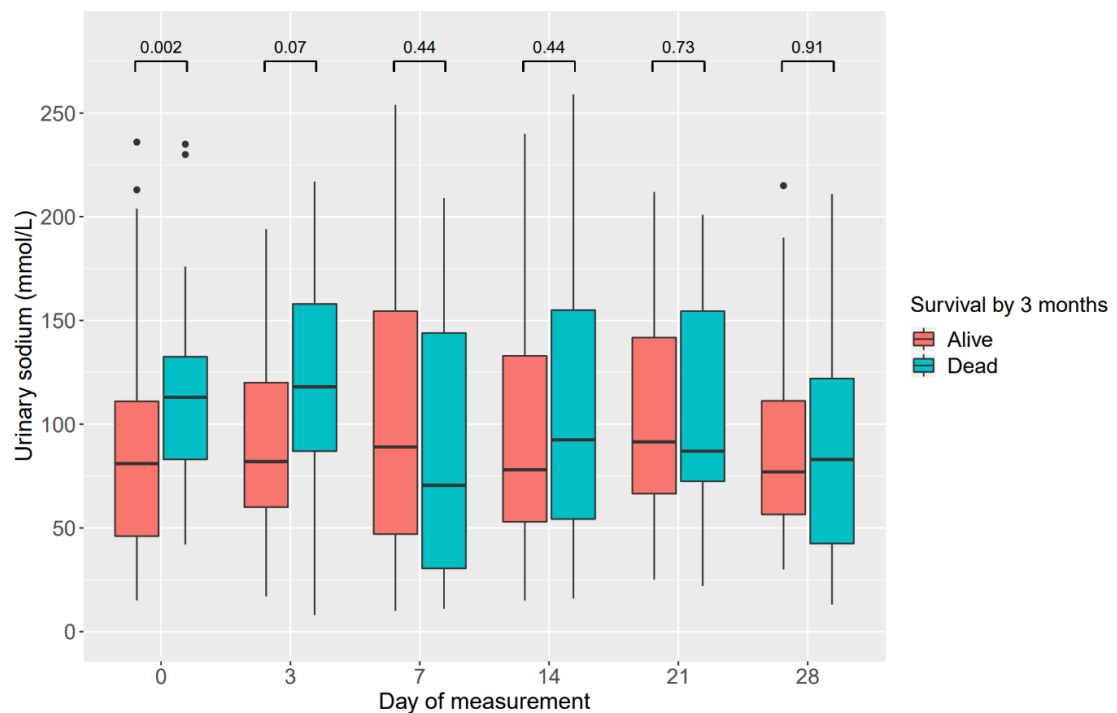
Day of assessment	Number of participants	Hypovolaemia by IVC ultrasound		Number of participants	No hypovolaemia by IVC ultrasound	
		Neurological complications (%)	No neurological complications (%)		Neurological complications (%)	No neurological complications (%)
0	18	3 (16.7%)	15 (83.3%)	38	8 (21.1%)	30 (78.9%)
3	13	0 (0%)	13 (100%)	24	3 (12.5%)	21 (87.5%)
7	13	2 (15.4%)	11 (84.6%)	17	5 (29.4%)	12 (70.6%)
14	15	1 (6.7%)	14 (93.3%)	15	3 (20.0%)	12 (80.0%)
21	12	3 (25.0%)	9 (75.0%)	23	7 (30.4%)	16 (69.6%)
30 *	34	2 (5.9%)	32 (94.1%)	3	1 (33.3%)	2 (66.6%)

* For IVC ultrasound, 'day 28' scans were performed at day 30 (+/- 1 day). For consistency throughout this results chapter they are again referred to as 'day 28' here. IVC=inferior vena cava.

5.3.11.4 Urinary sodium and clinical outcome

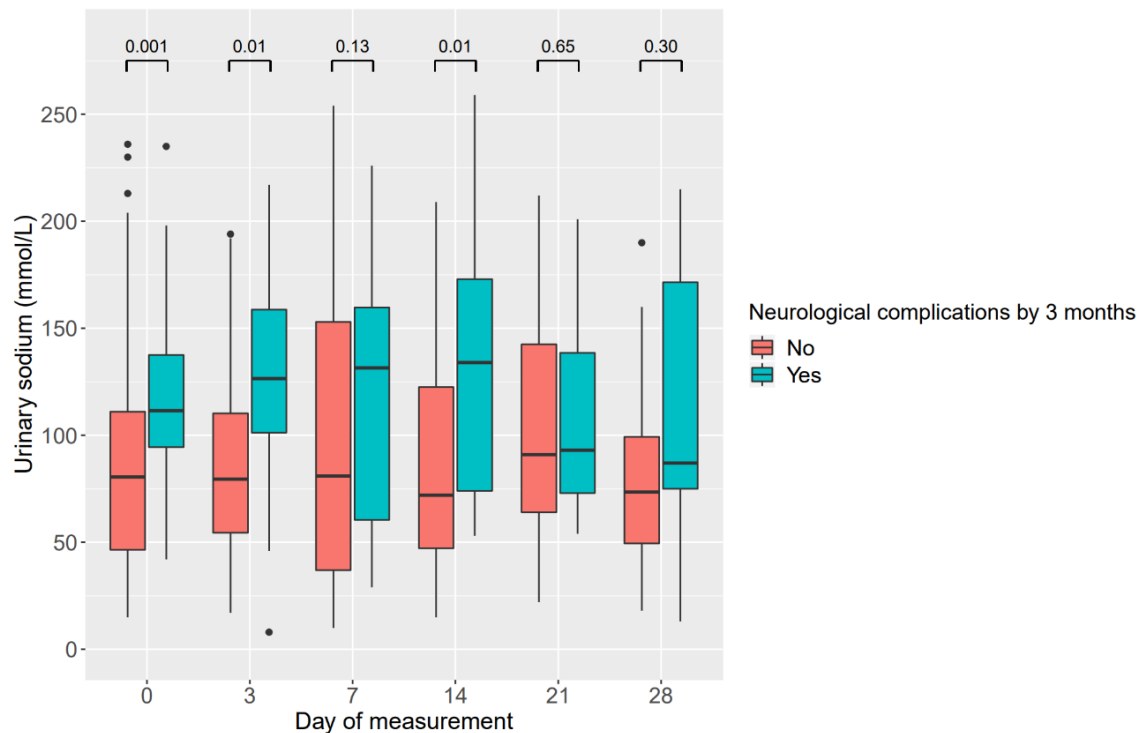
Clinical outcomes stratified by urinary sodium are shown in figures 5-19 and 5-20, respectively. Urinary sodium was significantly higher in participants who died by 3 months, at both baseline and day 3, but not thereafter. Urinary sodium was significantly higher in participants who experienced neurological complications by 3 months, at baseline, day 3, and day 14.

Figure 5-19: Urinary sodium stratified by survival by 3 months



For each individual boxplot, the central horizontal bar represents the median value. The box contains data between 3rd quartile (upper end of box) and 1st quartile (lower end of box). Vertical lines above and below each box extend to the most extreme data point that is within 1.5x the vertical height of the box. Dots represent individual data points. P values represent statistical comparison of groups by the Wilcoxon rank sum test.

Figure 5-20: Urinary sodium stratified by neurological complications by 3 months



For each individual boxplot, the central horizontal bar represents the median value. The box contains data between 3rd quartile (upper end of box) and 1st quartile (lower end of box). Vertical lines above and below each box extend to the most extreme data point that is within 1.5x the vertical height of the box. Dots represent individual data points. P values represent statistical comparison of groups by the Wilcoxon rank sum test.

5.4 Discussion

Hyponatraemia is commonly associated with TBM, although its pathogenesis, how it impacts upon clinical disease, and best management strategies, are poorly understood. Hyponatraemia may increase cerebral oedema, whilst its causative disease process also results in hypovolaemia, reduced cerebral perfusion, and worsening brain injury. Here I present a large and comprehensive descriptive analysis of hyponatraemia in HIV co-infected and HIV uninfected Vietnamese adults with TBM.

I begin by describing plasma sodium at baseline, stratified by HIV co-infection and by TBM severity grade. I then separate baseline hyponatraemic participants into mild, moderate and severe severity categories, describing baseline plasma sodium and its associated parameters by these severity groups. Next, I correlate lower baseline plasma sodium with markers of increased disease severity; higher CSF opening pressure, more neutrophilic CSF, and lower CSF/blood

glucose ratio, in addition to higher plasma cortisol which likely indicates increased host stress. I explore causality of hyponatraemia, initially at baseline, and then at further time points, using adapted causality-assessment criteria, followed by more exploratory methods. I then significantly correlate lower plasma sodium with increased urinary output, and with increased death by 3 months, at time points after baseline. Finally, I join together a clinical picture of hyponatraemia in TBM; low plasma sodium, low serum osmolality, high urinary sodium, and high urinary osmolality. Initially, SIADH appears more common a cause of hyponatraemia; however, as treatment progresses increasing urinary output heralds an increased risk of death. In this discussion I will now address these points in more detail.

I first demonstrate that hyponatraemia is a commonly associated abnormality of TBM, with hyponatraemia profound in almost 50% of cases in this study. Baseline plasma sodium was significantly lower with higher MRC TBM severity grade. Additionally, there were significant associations between lower plasma sodium, and higher lumbar CSF opening pressure, elevated baseline CSF neutrophil percentage, elevated absolute CSF neutrophils, and lower CSF/blood glucose ratio. These four parameters; MRC TBM severity grade, lumbar CSF opening pressure, CSF neutrophils, and CSF/blood glucose ratio, represent severity, ICP, and inflammation. The significant association between lower plasma sodium at baseline and each of these suggests hyponatraemia is a feature of more severe TBM disease and more severe intracerebral inflammation. Assessment of baseline plasma sodium by its severity, i.e. mild, moderate, or profound, demonstrated that profound hyponatraemia associated more frequently with HIV co-infection (vs. HIV uninfected), and with higher MRC TBM severity grade. Both HIV co-infection and high MRC TBM severity grade are association with excessive intracerebral inflammation,[18] indicating this inflammation as a possible mechanism of profound hyponatraemia.

Secondly, lower baseline plasma sodium was significantly associated with increased death by 3 months, and with neurological complications by 3 months, at all time points after baseline. Low plasma sodium after baseline appears to identify individuals with poor prognosis. A lack of significant association (of plasma sodium, with increased death or with increased neurological complications) at baseline may reflect the uncertain course and outcome of TBM disease when predicting at baseline; individuals with apparently stable disease at baseline may develop severe complications, and these individuals are often difficult to identify. This concept is further illustrated in the two illustrated individual participant datasets; plasma sodium is persistently low in the severe individual with poor outcome.

Thirdly, these baseline parameters identify a clinical pattern of hyponatraemia in TBM. Baseline plasma sodium significantly correlated with baseline serum osmolality, baseline urinary sodium, and baseline urinary osmolality. Interestingly urinary sodium levels appear to reduce when hyponatraemia becomes profound. It is possible that blood sodium-depletion eventually results in reducing urinary sodium, or alternatively that compensatory sodium-retention mechanisms are able to function when hyponatraemia becomes so severe. Interestingly a correlation was seen between baseline plasma sodium and baseline plasma cortisol. This may represent a stress response to TBM, with greater stress in more severe disease (where plasma sodium is lower). Whilst an Addisonian state may cause hyponatraemia (thereby associating low cortisol with low sodium), here it seems likely that plasma cortisol is responding to the hyponatraemia, rather than causing it.

Data suggested SIADH may cause hyponatraemia initially; however, causality analyses were complex, and most hyponatraemic participants were in fact polyuric (with variable intravascular volume depletion). Critically, 24-hour urinary outputs were significantly higher at all time points after baseline in participants who died by 3 months, and in participants who experienced neurological complications by 3 months, illustrating the importance of urinary output in clinical outcome

A tetrad of low plasma sodium, low serum osmolality, high urinary sodium and high urinary osmolality, followed by persistent hyponatraemia and very high urinary output in individuals with poor clinical outcome, describes a common set of events seen in clinical practice; rapid elevation in urine output heralds haemodynamic compromise, neurological deterioration, and poor outcome. This clinical picture moves away from the traditional method of focusing on assigning unique and single causality to hyponatraemia of TBM. This is often done to ascribe CSW or SIADH diagnoses; yet this is hugely challenging due to complicated criteria and subjective extracellular fluid assessments. The causality analyses performed in this manuscript illustrate the complexity of methodology and flaws in a causality-assignment approach.

I argue that using distinct labels of CSW or SIADH is not hugely beneficial to clinicians. What is more important is that the common model of TBM-hyponatraemia (low plasma sodium, low serum osmolality, high urinary sodium, high urinary osmolality, followed by persistent hyponatraemia and an increasing urinary output) is monitored for and recognised. A systematic approach to hyponatraemia assessment should be followed, with causes such as vomiting, adrenal insufficiency and drugs screened for, and excluded or addressed. Crucially, ascribing a diagnosis of SIADH, followed by initiation of a fluid restriction strategy (as is common for

SIADH in non-TBM disease), has potential for harm in large urine volume hyponatraemic states. High urine volumes and haemodynamic insufficiency associate with death in TBM. Volume replacement appears critical to maintain blood pressure and cerebral perfusion in these scenarios (although optimal sodium containing therapy is less certain).

This study has limitations. As discussed, any approach of allocating hyponatraemia causality (to CSW or SIADH) in TBM is likely too simplistic; both may occur in tandem, in sequence, or together with other causes such as vomiting. Hyponatraemia causality at baseline may not necessarily reflect ongoing causality. Analysis methods for assigning hyponatraemia causality are arbitrary and lack a strong evidence base. The use of '2 of 4' or '2 of 5' parameters to assign causality avoids a single erroneous result assigning erroneous hyponatraemia causality. Yet such an approach assumes equal weighting of contributing parameters (each scoring 1 point if present), and that when two parameters are present this is sufficient to assign hyponatraemia causality. In this study there were limitations in the data used to assign causality of hyponatraemia. IVC ultrasound data was not available for all participants, nor was a fluid balance clinical assessment. For this reason, hyponatraemia causality was assessed only in a smaller sub-population for whom all 'hypovolaemia' parameters; clinical, laboratory, fluid balance, IVC ultrasound, and urine output, were available. This may introduce bias; however, population comparison in this study suggested the population undergoing first causality assessment (n=34) was representative of the total study population. Even with a complete dataset, care must be taken in interpretation of parameters. Hypovolemia assessment is used as a proxy for extracellular fluid assessment and is not the same. Clinical signs used to assess hypovolaemia are subjective. Laboratory parameters such as haemoglobin and urea may only change at a late stage of hypovolaemia. Fluid balance may appear normal due to aggressive fluid resuscitation therapy (to match large urine outputs in a hypovolaemic individual). IVC ultrasound views may be challenging to obtain (when obscured by bowel gas and fat), and significant IVC variability may not necessarily reflect hypovolaemia.

In this study, when assigning non-CSW non-SIADH causes of hyponatraemia, an additional limitation was the lack of data recording diarrhoea at baseline; however, it is unlikely that diarrhoea would be a frequent ailment in TBM, and less so a cause of hyponatraemia. Finally, causality of hyponatraemia is assigned independently of administration of confounding sodium-containing therapies. Such therapies, including hypertonic 3% saline, may temporarily mask hyponatraemia, or cause rebound sodium-excretion effects after administration. For these reasons, a baseline assessment of hyponatraemia causality was exploratory only, and performed only where available data facilitated this.

An additional limitation of this study is that it is unable to link use of sodium-containing therapies to an effect on clinical outcome. Use of hypertonic saline 3% was more frequent in more severe TBM disease, given lower plasma sodium, for longer time periods, and worse neurological condition. Whether hypertonic saline 3% improves clinical outcomes in TBM cannot be answered by this data and requires a randomised controlled trial. Evidence supporting optimal sodium-containing therapies in TBM would be highly useful clinically and should be a future research priority. Reflecting the heterogeneity of individual participants is also a challenge for this study, where data is combined into, and compared between, groups. For this reason, two individual participants were shown in detail, to illustrate the complexities of analysing multiple sodium parameters and making patient decisions.

Strengths of this study are that it is large and contains baseline and longitudinal data describing plasma sodium and associated parameters, for adults with TBM across a variety of TBM disease phenotypes. As part of two clinical trials, demographic, baseline, and outcome data were collected, strictly following trial protocols and standard operating procedures.

In conclusion, this study describes plasma sodium and associated parameters during the first 30 days of anti-TB chemotherapy. Baseline plasma sodium was significantly lower with more severe TBM, higher lumbar CSF opening pressure, and more inflammatory CSF. After baseline, lower plasma sodium and higher 24-hour urinary outputs strongly predicted poor outcomes. The mechanism of hyponatraemia in TBM appears to be through renal loss of sodium (with polyuria), with data suggesting this process is driven by intracerebral inflammation and ICP. Hyponatraemia causality assessment criteria such as those used in this study may place insufficient importance on urinary volume, and lack an evidence base to separate ‘high’ urinary sodium of SIADH from ‘higher’ urinary sodium of CSW. Persistent hyponatraemia and rising urinary output should be closely monitored for, and fluid restriction avoided in most cases. Further data is urgently required to guide best management approaches for TBM-associated hyponatraemia.

5.5 Publications related to this chapter

The methods described within this study are published in the research protocols below:

1. Adjunctive dexamethasone for the treatment of HIV-uninfected adults with tuberculous meningitis stratified by *Leukotriene A4 hydrolase* genotype (LAST ACT): Study protocol for a randomised double blind placebo controlled non-inferiority trial

Joseph Donovan, Nguyen Hoan Phu, Le Thi Phuong Thao, Nguyen Huu Lan, Nguyen Thi Hoang Mai, Nguyen Thi Mai Trang, Nguyen Thi Thu Hiep, Tran Bao Nhu, Bui Thi Bich Hanh,

Vu Thi Phuong Mai, Nguyen Duc Bang, Do Chau Giang, Dang Thi Minh Ha, Jeremy Day, Nguyen TT Thuong, Nguyen Nang Vien, Ronald B. Geskus, Tran Tinh Hien, Evelyne Kestelyn, Marcel Wolbers, Nguyen Van Vinh Chau, Guy E. Thwaites
Wellcome Open Research 2018 Mar 20;3:32

2. Adjunctive dexamethasone for the treatment of HIV-infected adults with tuberculous meningitis (ACT HIV): Study protocol for a randomised controlled trial

Joseph Donovan, Nguyen Hoan Phu, Nguyen Thi Hoang Mai, Le Tien Dung, Darma Imran, Erlina Burhan, Lam Hong Bao Ngoc, Nguyen Duc Bang, Do Chau Giang, Dang Thi Minh Ha, Jeremy Day, Le Thi Phuong Thao, Nguyen TT Thuong, Nguyen Nang Vien, Ronald B. Geskus, Marcel Wolbers, Raph L. Hamers, Reinout van Crevel, Mugi Nursaya, Kartika Maharani, Tran Tinh Hien, Kevin Baird, Nguyen Huu Lan, Evelyne Kestelyn, Nguyen Van Vinh Chau, Guy E. Thwaites

Wellcome Open Research 2018 018 Jun 20;3:31

Chapter 6

Discussion

6.1 What were the goals of this thesis?

This thesis set the ambitious goal of improving diagnosis and understanding of the pathophysiology of TBM. TBM is a devastating neurological disease with high morbidity and mortality. Poor clinical outcomes from TBM are likely multifactorial. Clinically TBM is hard to recognise, with clinical presentation overlapping with that of other diseases. Diagnostic tools are insufficiently sensitive, contributing to delayed diagnosis. Multiple disease complications are hard to predict, and their best management is frequently uncertain with a limited evidence base to guide patient management in TBM. Improving diagnosis and further understanding pathogenesis may allow earlier recognition of both TBM and its complications, and pave the way for novel treatment approaches. This could lead to further clinical trials to ascertain best management practice in TBM.

As such, the goals of this thesis were divided into six chapters. Firstly, I have provided a general overview of TBM, discussing epidemiology, pathogenesis, diagnosis, monitoring and treatment. Whilst TBM data are not frequently published, several research groups are performing TBM research, therefore an up to date review of this field is necessary. In chapter two I have presented a prospective randomised diagnostic comparison study of Ultra and Xpert in TBM. The goal of this chapter was to assess for diagnostic superiority of the new Ultra cartridge over Xpert in TBM, i.e. provide an evidence base supporting use of Ultra in TBM in place of Xpert, consistent with current WHO advice.[167] In chapter three I have described the immunomodulatory influence of *S. stercoralis* co-infection on pathogenesis and intracerebral inflammation. Here my goal was to assess whether co-infection with the common helminth *S. stercoralis* was associated with less severe presenting phenotype, reduced neuroinflammation and improved clinical outcomes in TBM. Biological plausibility for this study derived from the known immunomodulation associated with helminth co-infection, and the detrimental effect of severe neuroinflammation in TBM. In chapter four I have evaluated whether ultrasound assessment of ONSD identifies severe disease, brain imaging abnormalities, and poor clinical outcomes in TBM. Given most neuro-complications of TBM have a final common pathway of elevated ICP, detection of this elevated ICP (by measurement of ONSD which becomes distended under elevated ICP) may have a role in TBM monitoring. In chapter five, I have

described the complexity of hyponatraemia in TBM, by longitudinal measurement of plasma sodium and its associated parameters. Very little is known of the role of plasma sodium in TBM (other than its association with both the disease, and poor outcome[35]). An increased understanding of plasma sodium in TBM, through detailed description, may provide data to support future clinical trials and treatment approaches. I conclude my thesis with this discussion (chapter six).

6.2 What did I find?

The road to improved diagnosis and understanding of pathophysiology is long, however, I believe the research contained within this thesis contributes towards advancement in this field. My key research findings, as described in the data chapters (chapters 2-5) of this thesis, were as follows. I was unable to show Ultra to have a 25% or more increased diagnostic sensitivity (vs. Xpert) for the detection of *M. tuberculosis* in CSF. Active *S. stercoralis* co-infection in TBM was significantly associated with reduced intracerebral inflammation and reduced neurological complications by 3 months. Using ultrasound, higher ONSD in TBM was significantly associated with abnormal brain imaging and with mortality by 3 months. Baseline plasma sodium was significantly lower in more severe TBM. Longitudinal sodium data during the first 30 days of anti-TB chemotherapy showed correlation between lower plasma sodium, lower serum osmolality and higher urinary osmolality, with persistent hyponatraemia and an increasing urinary output associated with individuals with poor clinical outcome.

6.2.1 GeneXpert MTB/RIF Ultra

Given the impact of Xpert on the TB diagnostic field in the past 10 years, and the promising early data describing Ultra for TBM diagnosis,[67] there were high hopes that Ultra would provide the next much-needed step forward for TBM diagnostics. However, in Vietnamese adults with suspected TBM, Ultra was not superior to Xpert for the identification of *M. tuberculosis* in CSF. This finding was disappointing, given Ultra cartridge improvements facilitate detection of lower bacillary numbers, and superior performance in detecting *M. tuberculosis* from sputum.[173] In a study of Ultra for TBM diagnosis in an HIV co-infected cohort in Uganda,[66] published on the same day as the data described in this thesis,[68] Ultra showed diagnostic superiority to Xpert for TBM diagnosis. At first glance, results from these two studies appear inconsistent. Taken together, how should they be interpreted by the clinician?

Firstly, our Ultra study in Vietnam, alongside the Ultra study from Uganda, serves to illustrate the importance of not relying on a sole study from a single site to infer global policy. CSF sampling volume and processing steps, host (genetics, HIV) and bacillary (lineage, variable genome *IS6110* copies) factors may all go some way to explaining why Ultra does not outperform Xpert in TBM in all settings.

Ultra may indeed be superior to Xpert; but not in all settings. Ultra's lower limit of detection of *M. tuberculosis* may have value in some cases, for example those with lower bacillary load who may be tipped from a negative result to a positive result by Ultra's superiority over Xpert. In our Ultra study, Ultra was superior to Xpert for TBM diagnosis in HIV uninfected cases (who are considered to have lower *M. tuberculosis* bacillary load than HIV co-infected cases), albeit this difference was not significant. In optimally processed CSF samples with high bacillary load, Ultra may be unable to improve diagnosis over Xpert.

Where does this leave the field of diagnostics in TBM? Certainly, this field is one of the most active in TBM research, perhaps the most active. Conventional molecular tests may be developed further, however whether further modifications can be applied to the Ultra cartridge to overcome the paucibacillary nature of CSF in TBM is far from certain. Additionally, Xpert and Ultra perform to a high level for the diagnosis of pulmonary TB, which makes up ~85% of global disease.[1] Once molecular tests have been optimised for pulmonary TB, further development may not proceed. CSF LAMP may be cheaper, quicker, and more suitable for local healthcare centres than Xpert and Ultra, however LAMP does not detect rifampicin resistance and data in TBM is limited. The new molecular assays Truenat MTB and Truenat MTB Plus (Truenat) (Molbio Diagnostics, Goa, India) have WHO approval for pulmonary TB[172], and will introduce competition into the field of molecular assays for TB diagnosis.

Non-confirmatory tests utilizing proteomics and metabolomics, which are becoming more accessible as technological advances continue, have potential for the identification of highly sensitive and specific signatures typical of TBM. Likewise, metagenomic next generation sequencing (mNGS) may provide a diagnostic breakthrough, if ways can be found to overcome the low *M. tuberculosis* bacillary load in CSF, which will invariably result in *M. tuberculosis* DNA copies in an amplified sample becoming overwhelmed by huge copy numbers of host DNA.

Until a 'magic bullet' test becomes available, the best diagnostic approach for TBM appears to be through utilization of multiple testing methods; reliance on a single diagnostic test is

dangerous in TBM, given none can rule out TBM when negative. All available information (clinical, CSF, imaging) should be combined with optimal sampling, processing, and technical expertise, a multiple-testing approach, plus clinical experience in diagnosis of this challenging disease.

6.2.2 *Strongyloides stercoralis* co-infection

Severe TBM is usually underpinned by excessive host inflammation, which through blockage of CSF flow and mass effect, elevates ICP and causes irreversible neurological injury. *S. stercoralis*, a helminth with global distribution, may be carried asymptotically or lead to fulminant disease complications. The immunomodulatory effect of helminth co-infection, including by *S. stercoralis*, is well recognised, however the impact upon TBM where excessive host inflammation is so detrimental had not previously been studied.

In chapter three I describe the effect of *S. stercoralis* co-infection on presenting severity, CSF parameters and cytokines, and neurological complications by 3 months, in TBM. *S. stercoralis* co-infection was indeed associated with less severe disease, reduced intracerebral inflammation, and improved outcome, as hypothesized. Association of *S. stercoralis* with reduced inflammation does not necessarily mean causation, however it seems more plausible that *S. stercoralis* is responsible for producing an improved phenotype and outcome, rather than less severe TBM providing an increased risk of acquiring *S. stercoralis*. Alternatively, an additional confounding factor may increase risk of both acquiring *S. stercoralis* co infection and developing less severe TBM.

S. stercoralis co-infection appears able to beneficially influence host inflammatory response to TBM. But how does it do this? Given adjunctive host directed therapies aim to control or at least minimise excessive inflammation of TBM with varied success, unpicking the mechanisms by which *S. stercoralis* mediates such beneficial effects may identify pathways that can be targeted with novel therapies. Progressing to such a host directed therapy may need detailed ‘omics data, with transcriptomic, proteomic, and metabolomic data potentially identifying differences in gene expression, translation, and molecules further downstream in the inflammatory process, between individuals with and without *S. stercoralis* co-infection.

Theoretically an alternative approach could be to ‘hijack’ helminth-associated immunomodulation; for example, use of non-pathogenic helminth antigen. Whether this would provide the same immunomodulatory response as *S. stercoralis* is of course unknown, as is

whether this would translate to clinical benefit to some or all individuals. This would require initial in-vitro studies measuring host immunomodulation upon stimulation with helminth antigen, to identify best antigen, and best dose, that correlate with an appropriate shift towards a Th2 host response to *M. tuberculosis* infection. Moving successful in-vitro data to human trials of TBM needs in-depth consideration of safety, given immunomodulation towards a Th2 response may be detrimental, not beneficial, in pulmonary TB, and may hasten progression from *M. tuberculosis* infection to TB. Numerous expensive therapeutic agents that are inaccessible to most individuals with TBM already exist, therefore benefit of the addition of a new agent to this list is uncertain. Additionally, reduction of host inflammation may indeed be detrimental for some individuals (who do not have an excessive neuroinflammatory response) with TBM. However, this point holds true for many current therapies (for example corticosteroids) where therapy is given to all or most individuals with benefit only expected in some.

Current therapies for TBM have not yet been optimised; both for killing of mycobacteria, and control of host inflammation. Further progress towards understanding the complex pathophysiology of TBM and its influencing factors (for example helminth co-infection), should aid development and refining of these therapies.

6.2.3 Optic nerve sheath diameter

Once a diagnosis of TBM has been made and treatments initiated, the clinical course is highly variable and often hard to predict. Therefore, monitoring to identify individuals who experience life threatening neuro-complications is important; yet gold standard invasive monitoring is infrequently performed in brain infection and is not accessible to most individuals with TBM who reside in resource-poor settings. Given most neuro-complications result in elevated ICP, detecting this raised ICP may be valuable. Further data are needed to support ICP-targeted therapies in TBM, yet prevention of further brain injury by instituting drainage of CSF (e.g. ventriculoperitoneal shunting) or mass reduction therapies (e.g. corticosteroids) upon the detection of hydrocephalus or tuberculomas, respectively, makes early detection of raised ICP enticing in TBM.

ONSD ultrasound may offer this early detection of raised ICP; a point-of-care tool, early to learn, and quick to perform. In this thesis I demonstrated that ONSD was significantly higher in TBM-affected individuals with abnormal brain imaging or who die by 3 months (vs. normal brain imaging, or survival by 3 months, respectively). This association between TBM severity and ONSD offers promise for ONSD ultrasound in TBM. However, important next steps must

be taken to show that ONSD ultrasound can be a useful clinical tool in TBM. Further research must demonstrate that the management steps taken upon detection of high ONSD result in improved clinical outcomes. This in turn requires ONSD thresholds to be set. i.e. what ONSD value should trigger a set of actions, and what should these actions be? In this thesis a receiver operating characteristic (ROC) curve analysis demonstrated ‘best’ ONSD cut-off values for predicting death by 3 months, with associated sensitivity and specificity. However, these sensitivity and specificity values (73% and 54%, respectively) are unlikely to be accurate enough to move forward with ONSD as a useful predictive tool at this point.

Additionally, upon recording an extremely high ONSD value, which strongly predicts death, what steps should be taken? Performing 3D brain imaging would likely be a useful next step, to characterise the disease processes involved. Hydrocephalus and tuberculomas represent disease processes that raise ICP and have recognised management approaches that may be tried. Surgical intervention for hydrocephalus would need neurosurgical support, in addition to a procedure, which may not be indicated in early stage TBM (ventriculoperitoneal shunts can block), or in those with poor prognosis unlikely to benefit from the procedure. Little evidence guides medical therapies for hydrocephalus in TBM. Inflammatory tuberculomas may benefit from corticosteroids, however no trial has shown corticosteroids to confer benefit for tuberculomas.

Despite uncertainty surrounding how to proceed after identification of a high ONSD value, this thesis has shown ONSD ultrasound to have promise as a monitoring tool in TBM. Consistency in scanning technique, with standardisation of measurement and interpretation, and development of teaching materials and guidelines, will help refinement of this technique, which may become a key part of TBM monitoring in the future. It now may be feasible to conduct a clinical trial where participants are randomised to standard care, or to daily ONSD ultrasound plus standard care. ONSD greater than a specific threshold (such as that threshold predicting death with 73% sensitivity) would result in 3D brain imaging being performed, followed by initiation of standard management strategies based upon 3D brain imaging findings. Clinical outcomes, survival, and neurological events would be measured.

6.2.4 Hyponatraemia

Hyponatraemia is common in TBM, yet why it occurs and what to do about it remain a mystery. The common clinical picture of hyponatraemia in TBM – as illustrated in this thesis; low plasma sodium, low serum osmolality and high urinary sodium, followed by persistent

hyponatraemia and an increasing urinary output, likely contributes to raised ICP. Hyponatraemia reduces oncotic pressure allowing free water to enter brain tissue, and it is possible this raised ICP effect may only serve to drive hyponatraemic pathology further. Knowledge of how to break this cycle, if indeed such a pathological cycle operates, would be valuable clinically. Low plasma sodium associates with severe TBM disease with poor outcome. Yet this does not automatically mean that the low sodium is the cause of poor outcome (rather it may be part of normal compensation), and no evidence exists to support correction of hyponatraemia for improved clinical outcomes. Administering large volumes of hypertonic saline may only serve to increase urinary sodium concentration with little benefit otherwise.

An improved understanding of hyponatraemia, as is offered by this thesis, may provide clues to best therapeutic approaches to hyponatraemia of TBM. The biggest question regarding plasma sodium in TBM seems to be ‘should we try to correct it’? Certainly, hyponatraemia is associated with increased mortality in TBM, but this does not mean correcting hyponatraemia improves survival, if indeed it is possible to correct it. What does seem clear in clinical practice is that the hypovolaemic state accompanying hyponatraemia does need correcting; the alternative would be haemodynamic instability. In this case should fluid resuscitation contain sodium? Conventional wisdom would say ‘yes’, to avoid diluting plasma sodium further. However conclusive data are needed.

A clinical trial comparing hypertonic saline therapies against conventional treatments would be valuable. Given the variety of management approaches, a multi-arm trial design may be most suitable. A trial arm could both dictate therapy (for example solely 3% hypertonic saline, or the local clinician’s choice given resources are likely to vary globally), in addition to targets (either a volume target [a defined correction of hypotension] or a volume plus sodium target [defined correction of hypotension, plus defined normalisation of plasma sodium]). All fluid therapies, plus inotrope use and complications would be recorded. Clinical outcomes would be measured.

6.3 Limitations of research

The research I have described within this thesis has limitations. Whilst limitations have been described in detail within each individual chapter, the limitation of blinded study drug (dexamethasone or placebo) at the time of writing requires re-discussion here. Three of my data chapters (chapters 3-5, inclusive) analyse data from the ACT HIV and LAST ACT clinical trials. These are randomised double blinded placebo controlled clinical trials of adjunctive dexamethasone for TBM. When devising my thesis questions, I tried to mitigate this effect of

blinded study drug, by either researching areas unlikely to be affected by the co-administration of dexamethasone or placebo, or by analysing clinical and sample data from before study drug was administered.

For example, CSF cytokines (chapter 3, *S. stercoralis*) were measured in CSF taken at baseline (i.e. before study drug was administered). In chapter 4, ONSD was measured longitudinally (during the first 30 days of anti-TB chemotherapy, i.e. after study drug was administered). However, a conclusion of association between ONSD and brain imaging remains true whether the participants had received dexamethasone or placebo. The biggest consideration for these data was clinical outcome by 3 months. As explained in individual thesis chapters, I believe it highly unlikely that administration of dexamethasone or placebo would affect the conclusions I have drawn. For example, if baseline *S. stercoralis* co-infection in TBM reduced neurological complications by 3 months, there is a theoretical chance that this was because more participants in this *S. stercoralis* group received corticosteroids (and corticosteroids were beneficial). However, more corticosteroids administration in the *S. stercoralis* group is less likely, due to the 1:1 randomisation nature of the trials. If those participants who received corticosteroids were then more likely to acquire *S. stercoralis*, then bias could be introduced, but this is implausible given study drug is first received after *S. stercoralis* sampling is performed. That these data chapters come from clinical trials (chapters 3-5) and a prospective brain infection study (chapter 2) is a strength of these data; well defined research protocols with standard operating procedures allowed precise research to be performed.

6.4 Next steps for the tuberculous meningitis research field

6.4.1 Status of the field

The TBM research community, and its scientific output, are growing. Clinical trial registry data show numerous ongoing and imminently starting clinical trials investigating anti-TB chemotherapy and/or adjunctive agents.[90] This will hopefully lead to evidence based management strategies in the near future. The Tuberculous Meningitis International Research Consortium, first convened in 2009,[10] now meets regularly, allowing sharing of ideas and dissemination of research. Elevating the research profile of TBM is important. TBM must be separated from ‘extrapulmonary TB’, a heterogeneous group which do not all possess the challenges associated with TBM. TBM guidelines should be updated, and updated WHO treatment guidelines for TB should specifically consider TBM.

6.4.2 Direction of the field

There are many directions in which research in the field of TBM could go, given the huge number of unanswered research questions. Focusing only on one research sub-area; patient management; TBM research gaps are quickly laid bare. Why is plasma sodium frequently low? Should we treat it, and if so how? Should we give corticosteroids, do they work for all patients, and what is the optimal corticosteroid dose, formulation and duration? How is supportive care best managed? What is the optimal temperature target, head-of-bed elevation angle, blood glucose target, blood transfusion threshold, and nutrition? Which drug best controls seizures with the fewest side effects and drug interactions? With hydrocephalus, when should an operation be done, on whom, by which method, and what is the best medical option if no surgery is available? Who gets paradoxical reactions and IRIS, how can they be predicted, and how should they be treated? Should we give aspirin for cerebral infarction, how much, and for how long? Many other sub-areas of TBM research have the same such research gaps.

Regarding my own future research, firstly I am keen to ensure high quality research translates into improved health outcomes globally. I would like to survey TBM treatment approaches before and after publication of ACT HIV and LAST ACT, to assess for changes in clinical practice associated with the published optimal treatment approach (corticosteroids or not). Secondly, I believe a ‘low-cost tool’ approach to brain infection can be beneficial; combining a published TBM ‘priorities’ checklist[322] with a clinical management bundle of easy-to-implement tools for improving patient outcome, including optimising head-of-bed angle and ventilation, temperature monitoring and normothermia maintenance, low-cost intracranial pressure monitoring (e.g. ultrasound), and hypotension/urine output monitoring; potentially developed and trialed through a global critical care network. I would like to explore translation of such tools to widely available smartphones, utilising their inbuilt technology, e.g. spirit level for head-of-bed elevation at home, and potentially colour intensity tools for rifampicin-urine or drug induced liver injury jaundice. Thirdly, more high-quality clinical trial data are needed for TBM, such as those proposed above (ONSD for raised ICP detection, and comparison of hyponatraemia management strategies), and those that address the TBM research gaps listed above.

The reality that there are many research gaps illustrates not only that data are limited in TBM, but also the exciting direction that the TBM research field can move in. The thought that some (or all) of these research questions can be answered, and that outcomes of a disease that kills up

to 50% of 100,000 cases each year can be improved, is inspiration to continue scientific research into TBM.

6.5 Conclusion

I believe that the research contained within this thesis contributes to the global efforts to improve diagnosis and understand pathogenesis of TBM, and ultimately improve upon the dire clinical outcomes currently seen with this disease. Yet, I recognise the limitations of my research, and the huge number of research gaps that must be addressed to allow myself and others to continue to make progress in this field. Fortunately, senior leading researchers are driving this field forward, supported by researchers who are developing their research skills, and increasing their knowledge and experience of TBM. I have learned an enormous amount during my PhD, and I am extremely grateful to those individuals who have supported me, inspired me, nurtured my writing, taught me how to answer questions, and more importantly, ask questions. I intend to stay active in the TBM research field and build on the research I have performed to date.

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Appendix A

Diagnostic criteria for TBM [69]

		Diagnostic score
Clinical criteria	(Maximum category score=6)	
	Symptom duration of >5 days	4
	Systemic symptoms suggestive of tuberculosis (one or more of the following): weight loss (or poor weight gain in children), night sweats, or persistent cough for >2 weeks	2
	History of recent (within past year) close contact with an individual with pulmonary tuberculosis or a positive TST or IGRA (only in children <10 years of age)	2
	Focal neurological deficit (excluding cranial nerve palsies)	1
	Cranial nerve palsy	1
	Altered consciousness	1
CSF criteria	(Maximum category score=4)	
	Clear appearance	1
	Cells: 10-500 per μ l	1
	Lymphocytic predominance (>50%)	1
	Protein concentration >1 g/L	1
	CSF to plasma glucose ratio of less than 50% or an absolute CSF glucose concentration less than 2.2mmol/L	1
Cerebral imaging criteria	(Maximum category score=6)	
	Hydrocephalus	1
	Basal meningeal enhancement	2
	Tuberculoma	2

	Infarct	1
	Pre-contrast basal hyperdensity	2
Evidence of tuberculosis elsewhere	(Maximum category score=4)	
	Chest radiograph suggestive of active tuberculosis: signs of tuberculosis=2; miliary tuberculosis=4	2 or 4
	CT/ MRI/ ultrasound evidence for tuberculosis outside the CNS	2
	AFB identified or <i>Mycobacterium tuberculosis</i> cultured from another source-i.e., sputum, lymph node, gastric washing, urine, blood culture	4
	Positive commercial <i>M. tuberculosis</i> NAAT from extra-neural specimen	4

Diagnostic criteria based on total score:

Possible TBM: score 6-9 (if no brain imaging) or 6-11 (if brain imaging)

Probable TBM: score >9 (if no brain imaging) or >11 (if brain imaging)

Definite TBM: acid-fast bacilli seen in CSF or *M. tuberculosis* cultured or detected by commercial NAAT in CSF

Appendix B

Stool concentration method

The currently recommended stool concentration method uses 1g of faeces, with 10% formalin in water added to emulsify, plus a surfactant (0.1% Triton X100) when using ethyl acetate as a solvent.[203] The stool sample is sieved using a pore (size $\leq 0.5\text{mm}$), vortexed for at least 15 seconds, and then centrifuged at 3000rpm for 3 minutes.[203] Allen et al[323] described further modifications to the simplified version[324] of Richie's original method.[325] The concentration method used by Allen et al[323] differed from that used by Ridley[324] in its use of formalin in water, in place of formol saline, and centrifugation at 3,000rpm instead of 2,000rpm. These modifications led to increased *S. stercoralis* parasite detection.[324] The method by Allen et al [323] is largely similar to that recommended today.[203] A study of 200 returned questionnaires from parasitology laboratories looked at variation in the stool concentration method, specifically, formalin diluted in water vs. formalin diluted in saline; sieve pore size during filtration, the use of ethyl acetate and Triton X, or ether alone, and centrifugation speed.[203] The study authors found formalin in water was significantly more effective than formalin in saline ($p \leq 0.005$ for recovery of parasites), a sieve pore size of $425\mu\text{m}$, (as per the Ridley-Allen Method) gave the best recovery of parasites, ethyl acetate and Triton X were superior to ether alone, and the recovery of parasites was greatest if the samples were centrifuged for 3,500rpm for 10 minutes.[203]

Appendix C

Additional stool *S. stercoralis* larval detection methods

In the agar plate culture method, linear bacterial colonies originating from a stool sample placed in the centre of an agar plate reveal the presence of migrating *S. stercoralis* larvae, which can then be extracted and confirmed.[326] Additionally, stool culture methods have been developed which exploit the ability of *S. stercoralis* to enter a free living development cycle.[240] The Harada-Mori and Baermann methods use filter paper and mesh, respectively, to capture *S. stercoralis* larvae after they move from a stool sample to warm water.[240]

Appendix D

Treatment of uncomplicated strongyloidiasis

1. Thiabendazole and ivermectin

Introduced in 1963, thiabendazole was the first line treatment for strongyloidiasis for many years; however ivermectin has now taken its place as the drug of choice.[192] Ivermectin binds to glutamate gated chloride ion channels of parasite neuronal cells, leading to hyperpolarisation and death.[190] Ivermectin is taken orally, and is generally well tolerated, with mild adverse reactions (for example pruritus, fever, rash, myalgia, and headache) usually occurring during the first 3 days after treatment.[327]

Clinical trials have been unable to show a benefit in parasitological cure of single dose 200µg/kg ivermectin over thiabendazole 50mg/kg daily, however ivermectin was associated with a better side effect profile making it a more appealing choice of therapy.[180,211–213] Bisoffi et al found significantly more mild-moderate side effects with thiabendazole than with single dose ivermectin (73.1 vs. 20.9%, respectively).[212] In two studies, a third intervention arm; two consecutive days of ivermectin at 200µg/kg per day, was also assessed.[180,211] A systematic review of 244 strongyloidiasis case reports found no significant difference in patient outcome between those treated with ivermectin and thiabendazole.[328] One of these studies found two days of ivermectin to result in improved parasitological cure vs. single dose ivermectin (n=35, cure=100% vs. n=22, cure=77%, respectively).[180] A further study comparing albendazole with both single dose ivermectin and with two doses of oral ivermectin 200µg/kg given 2 weeks apart, did not find superiority of the two-dose ivermectin regimen over a single ivermectin dose.[209] Whilst the benefit of ivermectin over thiabendazole appears to be through its superior side effect profile alone, the addition of a second dose of ivermectin has no clear benefit. However, recently a multicentre open-label randomised controlled trial compared single dose ivermectin 200µg/kg with four ivermectin 200µg/kg doses given on days 1,2, 15 and 16.[215] Clearance of *S. stercoralis* at 12 months was the primary endpoint. The multiple dosing regimen was less well tolerated and did not improve clearance of *S. stercoralis*, when compared with the single dose regimen. A single ivermectin 200µg/kg dose appears sufficient for uncomplicated strongyloidiasis.

2. Albendazole and ivermectin

The benefit of ivermectin over albendazole for the parasitological cure of strongyloidiasis has been demonstrated in multiple clinical trials, with parasitological cure rates of single dose

ivermectin and 3-7 day albendazole regimens reported as 83 vs. 38%, [206] 83 vs. 43%, [207] 98.7 vs. 78.7%, [208], and 96.8 vs. 63.3%. [209] In a Japanese study a fixed 6mg ivermectin dose led to higher parasitological cure than 3 days of 400mg/day albendazole. [329] Ivermectin has also shown superiority for strongyloidiasis treatment over higher dose albendazole therapy; 800mg for 7 days. [210]

A 2016 Cochrane review of strongyloidiasis treatment (7 trials, 1147 participants, 1994-2011) concluded superior parasitological cure with ivermectin compared to albendazole, no difference in cure with ivermectin compared to thiabendazole, (however more adverse events with thiabendazole), and no increase in cure when a second dose of ivermectin was added to a first dose of ivermectin. [214] This recommendation is supported by the recent trial [215] comparing single dose, and four dose, ivermectin.

The role of combination therapy (ivermectin plus albendazole) is not clear, and has only been described in case report form. [330]

3. Confirmation of *S. stercoralis* cure

A hallmark of most trials to date is the confirmation of cure by stool microscopy, a test with low sensitivity. Confirmation of cure is challenging, however new stool PCR tests potentially offer a new way to assess the effectiveness of drug therapy for strongyloidiasis. In a recent prospective study, 48 patients were diagnosed and treated (200µg/kg orally daily for two days, repeated after 2 weeks) for *S. stercoralis* infection in Argentina. [331] Participants were resident in areas considered to be non-endemic to avoid future positive tests being attributed to re-infection, and parasite re-exposure through travel was excluded at follow up appointments. Of the 21/48 (44%) participants who returned for follow up (followed up for a median of 730 days), 14/21 (67%) had parasitological reactivation (agar plate culture or fresh stool larvae), 9/21 (43%) had clinical reactivation (6/21 had both parasitological and clinical reactivation), and interestingly 21/21 had a positive stool PCR test. Corticosteroid use was reported in 6/21 cases. Ivermectin appeared poor at eradicating *S. stercoralis*, which may persist despite drug therapy.

Appendix E

Prophylaxis against *S. stercoralis* infection

Prophylaxis for *S. stercoralis*-negative individuals undergoing corticosteroid therapy has been trialled without success.[332] Patients with haematological malignancies or benign conditions requiring corticosteroids were randomised to 25mg/kg thiabendazole orally twice daily for two days repeated monthly, or to placebo, after three negative stool samples for *S. stercoralis*. Thiabendazole was not superior to placebo in reducing the development of strongyloidiasis, and significantly more abdominal pain events occurred in the thiabendazole group.[332] Whether ivermectin would represent an effective prophylactic agent is unknown, and has not been trialled.

Appendix F

Guidelines for treatment of tuberculous meningitis [41,85]

1. First line treatment

Rifampicin (10mg/kg/24hrs; maximum 600mg), isoniazid (5mg/kg/24hrs; maximum 300mg), pyrazinamide (25mg/kg/24hrs; maximum 2g) and ethambutol (20mg/kg/24hrs; maximum 1.2g) are given for for at least the first 2 months of treatment.

Pyrazinamide will then be stopped and rifampicin, isoniazid and ethambutol (at the same doses) will then be given until at least 12 months anti-tuberculosis treatment in total has been given. If pyrazinamide cannot be given for at least 2 months (for example, because of drug-induced toxicity), then total treatment should be at least 12 months.

2. Isoniazid-resistant tuberculosis

Option 1: Follow the standard regimen above, but replace isoniazid with levofloxacin (20mg/kg/24 hrs; maximum 1000 mg/day). Pyrazinamide can be used throughout treatment in those with more severe disease who are responding slowly.

Option 2: Stop isoniazid and treat with rifampicin, ethambutol and pyrazinamide for the entire 9-12 months of treatment. This option is not suitable for those with confirmed ethambutol resistant bacteria; these participants should be treated with option 1.

3. Multi-drug resistant tuberculosis

Second line treatment for MDR TBM should be given as soon as possible, following National guidelines and local policies.

Appendix G

Individual studies comparing ONSD with invasively measured ICP

Geeraerts et al measured ONSD in 31 patients with severe TBI and found ONSD was significantly higher in patients who developed raised ICP in the 48 hours after trauma ($6.3 \pm 0.6\text{mm}$) vs. patients with normal ICP ($5.1 \pm 0.7\text{ mm}$). [333] A further study in neurocritical care patients assessed simultaneous measurement of ONSD by ultrasound and invasively monitoring ICP. [252] ONSD measurements were performed 78 times in 37 patients, with the causes of raised ICP as follows; severe TBI (n=22), subarachnoid haemorrhage (n=6), intracranial hematoma (n=8) and stroke (n=1). A ROC curve for ICP > 20 mmHg demonstrated that ONSD predicted raised ICP with a best cut-off value of 5.86 mm (sensitivity 95%, specificity 79%). Kimberley et al performed 38 ocular ultrasounds in 15 patients with TBI (n=4), and intracranial haemorrhage (n=11). [334] A ROC curve for ICP > 20 mmHg demonstrated that ONSD predicted raised ICP with a best cut-off value of 5.0mm (sensitivity 88%, specificity 93%). [334] A cut-off value of 4.5mm gave a sensitivity of 100%, but a specificity of only 63%. Moretti et al performed 53 ONSD measurements prior to invasive ICP monitor placement in patients with intracranial haemorrhage and found that ONSD predicted ICP > 20 mmHg with a best cut-off value of 5.2mm (sensitivity 94%, specificity 76%). [335] A second study by Moretti et al measured ONSD 94 times in 63 patients with primary intracerebral haemorrhage (n=29) or subarachnoid haemorrhage (n=34). [336] ONSD predicted ICP > 20 mmHg with a best cut-off value of 5.2mm (sensitivity 93%, specificity 74%). [336] Soldatos et al measured ONSD in 89 critical care patients with brain trauma (n=62) and in control patients with no neurological injury (n=27). [266] ONSD predicted ICP > 20 mmHg with a best cut-off value of 5.7mm (sensitivity 74%, specificity 93%). [266] Rajajee et al performed 576 ONSD measurements in 65 patients with the following pathologies; subarachnoid haemorrhage (n=30), intracerebral haemorrhage (n=11), TBI (n=11), brain tumour (n=5), ventriculoperitoneal shunt malfunction (n=5), ischemic stroke (n=1), cerebral venous sinus thrombosis (n=1), and acute liver failure (n=1). [250] ONSD predicted ICP > 20 mmHg with an optimal cut-off value of 4.8mm (sensitivity 96%, specificity 94%). [250]

In 110 Chinese patients with varying neurological diagnoses (diagnoses not stated) ONSD was compared with lumbar opening pressure. [337] An ONSD > 5.6mm predicted ICP > 20cmH₂O with 86% sensitivity and 73% specificity. In a large prospective observational study in the United Kingdom, 445 ONSD measurements were performed in 64 patients with TBI, intracerebral haemorrhage or stroke, and compared against invasively measured ICP

measurements.[338] Mean ONSD in those who survived was 5.11 mm compared with 5.71 in those who died. In a prospective study of Malaysian neurosurgical patients an ONSD of 5.2mm detecting an ICP > 20mmHg with 96% sensitivity and 80% specificity.[339] In a Korean study an optimal cutoff for ICP detection was shown to be 5.6mm.[340]

Appendix H

Validation of ONSD using brain imaging consistent with raised ICP

Subsequent to correlation of ONSD with invasively measured ICP, research groups have set out to establish ONSD thresholds that define raised ICP in specific populations, using alternative comparator tests to gold standard invasive measurements for defining raised ICP. 3D brain imaging, such as CT or MRI, has emerged as such a comparator test. Whilst 3D brain imaging is unable to measure ICP, imaging features consistent with raised ICP can be noted. A study by Lee et al noted that most studies evaluating ONSD measured by ultrasound for ICP detection had been performed in European Caucasian populations, therefore the authors set out to demonstrate the role of ONSD ultrasound in a South Korean population.[341] A significant difference was seen between average ONSD values of patients with raised ICP and those of a healthy control group (median ONSD: 5.9mm, IQR 5.8-6.2mm, vs. 4.9mm, IQR 4.6-5.2mm, respectively, $p < 0.001$).[341] ONSD predicted $ICP > 20$ mmHg with an optimal cut-off value of 5.5mm (sensitivity 99%, specificity 85%).[341] The authors defined raised ICP based on brain MRI appearances rather than invasive intracranial measurements, with MRI brain imaging and ONSD ultrasound performed within 1 hour of each other. MRI findings consistent with one of the following criteria were used to define raised ICP: significant brain oedema, midline shift, compression of ventricle or basal cistern, effacement of sulci, insufficient grey/white differentiation or transfalcal herniation,[341] using criteria developed by Miller et al.[259]

Miller et al originally developed a scoring system based on five CT brain imaging characteristics, with these characteristics chosen by the authors based on literature review and consultation with study investigators.[259] In their study, patients scored 1, 2 or 3 points for significant brain oedema, midline shift, compression of ventricle or basal cistern, effacement of sulci, insufficient grey/white differentiation or transfalcal herniation. Whilst this analysis indicated that initial ICP could not be predicted by the presence of these CT characteristics, a univariate linear regression analysis demonstrated a significant correlation between average initial ICP and the average radiologic score of four of five of these CT brain characteristics, with the fifth characteristic, basilar cistern size, trending toward linear association with average initial ICP.[259] More evidence is clearly required to directly link these CT scan characteristics to raised ICP, however these CT brain criteria, or adapted versions of them, have subsequently been used as surrogates for raised ICP. Goel et al measured ONSD in 100 patients with head injury, and correlated ONSD with CT brain imaging (CT brain and ONSD ultrasound were performed within 20 minutes of each other).[342] CT imaging was considered positive for

raised ICP if the following were present; significant oedema, midline shift of 3mm or more, mass effect, effacement of sulci, collapse of ventricles or compression of cisterns.[342] Blaivas et al performed ONSD ultrasound in 35 patients with suspected raised ICP caused by intracranial haemorrhage.[343] Patients were grouped by the presence of CT brain findings consistent with raised ICP; mass effect with a midline shift 3 mm or more, a collapsed third ventricle, hydrocephalus, effacement of sulci with significant edema, and abnormal mesencephalic cisterns. An average (across left and right eyes) ONSD of > 5mm was taken to be consistent with raised ICP. The mean ONSD of patients with CT brain imaging consistent with raised ICP (n=14) was 6.27mm (95% CI 5.6-6.9), compared with a mean ONSD for patients without these CT findings of 4.42mm (95% CI 4.2-4.7), with comparison between these groups statistically significant (p=0.001).[343] All 14 patients with average ONSD > 5mm had CT brain imaging consistent with raised ICP.[343] A study of 753 patients with severe non penetrating head injury found that CT findings indicating cerebral herniation (particularly abnormal mesencephalic cisterns and midline shift) were strongly associated with a risk of elevated ICP.[344]

Appendix I

Methods for recording and averaging ONSD

In a study of 115 adult patients at risk of raised ICP there was no significant difference between lateral and transverse measurements taken from each eye, and no significant difference between measurements taken from left vs. right eye, with a conclusion drawn that ONSD measurements can be taken in either eye, in either orientation.[345] However, in their study of 100 patients with non-TBI, Komut et al found that in those with unilateral pathology (n=45), ONSD was significantly higher when measured in the eye on the side of the affected brain hemisphere, compared with measurement on the side of the unaffected hemisphere.[273] In published literature, methods of measuring, repeating and averaging differ widely. Published methods include performing a sagittal and transverse measurement for each eye and averaging for that eye,[252,336] repeating sagittal and transverse measurements for each eye and then averaging for each eye,[78,268,334] and repeating measurements for each eye and using the highest value recorded for each eye.[273]

Appendix J

Evidence supporting IVC ultrasound in fluid assessment

The use of IVC ultrasound in mechanically ventilated patients is largely based on two studies[346,347] published in 2004.[348] Barbier et al used IVC distensibility (dIVC), calculated as end-inspiratory IVC diameter minus end-expiratory IVC diameter, divided by end-expiratory IVC diameter, multiplied by 100 to express the value as a percentage.[346] A dIVC of 18% or above indicated a volume status responsive to intravenous fluid with 90% sensitivity and specificity.

Feissel et al defined IVC diameter variation (Δ IVC) as $[(D_{\max} - D_{\min}) / (D_{\max} + D_{\min}) / 2]$ and found a Δ IVC value of 12% discriminated between responders and non-responders to intravenous fluid, with a positive predictive value of 93% and a negative predictive value of 92%.[347] Despite the use of different calculation methods between the studies, changes in measured IVC were shown to correlate with fluid responsiveness, in patients perfectly synchronised with a mechanical ventilator in whom ventilation was predictable.[346,347]

A study of 40 spontaneously breathing patients with circulatory failure due to sepsis (n=24), haemorrhage (n=11) or dehydration (n=5) investigated whether IVC diameter variation predicted fluid responsiveness in these non-mechanically ventilated patients.[349] The authors measured IVC using M-mode ultrasound, and used a calculation similar to Barbier et al after accounting for maximal IVC occurring in expiration in spontaneously breathing patients $[(D_{\max} - D_{\min}) / D_{\max} \times 100]$ to calculate the IVC variation. In this study 20 (50%) patients were fluid responsive, with IVC variation >40% during respiration usually associated with fluid responsiveness (70% sensitivity, 80% specificity).[349]

Further studies have supported the use of IVC collapsibility for fluid resuscitation in critically ill patients who are spontaneously ventilated. Corl et al enrolled 124 spontaneously breathing patients with acute circulatory failure, and compared IVC ultrasound measurements to cardiac output fluid responsiveness (defined by a 10% increase in cardiac index after a 500mls intravenous fluid bolus, measured by a Non-Invasive Cardiac Output Measurement [NICOM] device).[350] An optimal caval index (cIVC) of 25% was identified (where $cIVC = [IVC \text{ expiratory diameter} - IVC \text{ inspiratory diameter}] / IVC \text{ expiratory diameter} \times 100$), with 87% sensitivity and 81% specificity.[350] Preau et al compared IVC collapsibility to stroke volume index increase after volume expansion with 500mls 4% gelatin, in 90 spontaneously ventilated patients.[351] A relevant increase (defined as $\geq 10\%$ increase in stroke volume index) was

recorded in 56% patients, and when IVC collapsibility index was superior or equal to 48%, this relevant fluid responsiveness was predicted with 84% sensitivity and 90% specificity.[351]

Appendix K

Serum osmolality storage

Serum osmolality testing is not available at HTD where patient samples are taken; serum must be frozen and stored for future testing. Recent studies have provided an evidence base for storage of serum osmolality samples.[311,352,353] Assuming immediate sample centrifugation occurs, both serum and plasma have been shown to be stable for 7 days at room temperature, and for at least 4 weeks at standard refrigeration temperatures (2-8⁰C), where a change >2.6% (2 standard deviations from the mean) was considered significant.[352] In this study an osmometer (3320 Osmometer, Norwood, MA) was used for osmolality measurements.

A study by Zhang et al found that time to separation from the clot in spun samples was important for the stability of glucose, a critical component of osmolality, with the authors recommending separation of serum from clot within 3 hours.[354] Deep freezing of samples may be more beneficial than standard freezing. In a study assessing freezing temperatures 25 patients, blood was withdrawn and stored as serum, as plasma in EDTA, and in lithium heparin tubes.[353] Storage temperatures were 22⁰C, 7⁰C, -21⁰C and -78⁰C, with analysis at 1, 3, 14, 21, and 56 days of storage. Sample testing was by Mikro-Osmometer Automatic, type 13–Autocal (Roebbling, Berlin, Germany). Whilst storage at 22⁰C and 7⁰C were associated with progressive rises in osmolality, samples stored at -21⁰C were associated with a decrease in osmolality. Samples stored at -78⁰C demonstrated stable osmolality testing at up to and including 56 days, both as serum and plasma.[353] The authors recommended that samples stored at -78⁰C should be thawed quickly (5 mins at 37⁰C) in a water bath, as a slow thawing process (12 hour defrost at 7⁰C) led to an increased in measured osmolality.[353]

Appendix L

Publications from this thesis

Wellcome Open Research

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STUDY PROTOCOL

REVISOR Adjunctive dexamethasone for the treatment of HIV-infected adults with tuberculous meningitis (ACT HIV): Study protocol for a randomised controlled trial [version 2; referees: 1 approved, 2 approved with reservations]

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Abstract

Background: Tuberculous meningitis (TBM) is the most severe form of tuberculosis. Co-infection with HIV increases the risk of developing TBM, complicates treatment, and substantially worsens outcome. Whether corticosteroids confer a survival benefit in HIV-infected patients with TBM remains uncertain. Hepatitis is the most common drug-induced serious adverse event associated with anti-tuberculosis treatment, occurring in 20% of HIV-infected patients. The suggested concentration thresholds for stopping anti-tuberculosis drugs are not evidence-based. This study aims to determine whether dexamethasone is a safe and effective addition to the first 6-8 weeks of anti-tuberculosis treatment of TBM in patients with HIV, and investigate alternative management strategies in a subset of patients who develop drug induced liver injury (DILI) that will enable the safe continuation of rifampicin and isoniazid therapy.

Methods: We will perform a parallel group, randomised (1:1), double blind, placebo-controlled multi-centre Phase III trial, comparing the effect of dexamethasone versus placebo on overall survival in HIV-infected patients with

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TBM, in addition to standard anti-tuberculosis and antiretroviral treatment. The trial will be set in two hospitals in Ho Chi Minh City, Vietnam, and two hospitals in Jakarta, Indonesia. The trial will enrol 520 HIV-infected adults. An ancillary study will perform a randomised comparison of three DILI management strategies with the aim of demonstrating which strategy results in the least interruption in rifampicin and isoniazid treatment. An identical ancillary study will also be performed in the linked randomised controlled trial of dexamethasone in HIV-uninfected adults with TBM stratified by LTA4H genotype (LAST ACT).

Discussion: Whether corticosteroids confer a survival benefit in HIV-infected patients remains uncertain, and the current evidence base for using corticosteroids in this context is limited. Interruptions in anti-tuberculosis chemotherapy is a risk factor for death from TBM. Alternative management strategies in DILI may allow the safe continuation of rifampicin and isoniazid therapy.

Keywords

Tuberculous meningitis, HIV, Dexamethasone, Drug-induced liver injury, LTA4H, Adrenal suppression, Diabetes, Strongyloides, Hyponatraemia

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REVISED Amendments from Version 1

The updated version clarifies aspects of anti-tuberculous and anti-retroviral therapy, answers reviewer questions regarding the ancillary DILI study, and describes how brain imaging is managed outside of the ancillary imaging study. All reviewer comments are addressed in the 'Note to Referees' section.

See referee reports

Introduction

Background

Mycobacterium tuberculosis causes approximately 10.4 million new cases of tuberculosis and 1.5 million deaths annually, with an additional 0.4 million deaths in individuals co-infected with human immunodeficiency virus (HIV)¹. Tuberculous meningitis (TBM) is the most severe form of tuberculosis, killing around 30% of all sufferers despite appropriate anti-tuberculosis chemotherapy². TBM is especially common in young children and in those with advanced immunodeficiency secondary to HIV, and is characterised by a slowly progressive meningo-encephalitis with necrotising granulomatous inflammation predominantly affecting the basal meninges.

Treatment of TBM

Rifampicin, isoniazid, pyrazinamide and ethambutol are recommended in current international guidelines for the treatment of drug-susceptible TBM, in adults with or without HIV, with treatment recommended for 9–12 months^{3,4}. The treatment of drug resistant TBM is more challenging. Adjunctive anti-inflammatory treatment with corticosteroids (dexamethasone) has been shown to improve survival in TBM, in predominantly HIV-uninfected individuals in a small number of trials⁵.

Complications of TBM

Neurological complications of TBM

Hydrocephalus, stroke, and tuberculoma formation are important complications of TBM⁶. They generally present within the first 3 months of treatment and can be fatal if not detected and treated quickly. Little evidence exists to help guide the management of these complications, which are common in HIV-infected and uninfected TBM patients of all ages⁷. In untreated HIV-infected patients with TBM, contemporaneous anti-tuberculosis treatment and anti-retroviral therapy (ART) can result in neurological inflammatory complications secondary to ART-driven IRIS⁸. Tuberculoma and spinal radiculomyelitis are the commonest IRIS complications described.

Drug induced liver injury (DILI)

Hepatitis is the most common drug-induced serious adverse event associated with anti-tuberculosis treatment, occurring in approximately 10% of HIV-uninfected and 20% of HIV-infected patients⁹. Almost all episodes occur in the first 3 months of anti-tuberculosis therapy, a critical time in TBM treatment. Rifampicin, isoniazid, and pyrazinamide can all cause DILI, although determining which drug is responsible in individual patients can be difficult. Anti-tuberculosis DILI is widely defined as elevation of blood transaminase concentrations ≥ 3 times the upper

limit of normal (ULN) with symptoms, or ≥ 5 times the ULN without symptoms⁴. US Centers for Disease Control and Prevention guidelines for pulmonary tuberculosis suggest stopping all anti-tuberculosis drugs in DILI until transaminase concentrations have returned to <2 times the ULN, followed by sequential re-introduction⁴, however this approach is probably unsafe in those with TBM. We have previously shown that interruptions in first-line anti-tuberculosis chemotherapy for any reason is an independent risk factor for death from TBM¹⁰. The suggested concentration thresholds for stopping anti-tuberculosis drugs are not evidence-based. The optimal strategy for managing DILI in TBM is uncertain⁴. The majority of asymptomatic rises in transaminases (even those >5 times the ULN) will resolve spontaneously, therefore higher thresholds for stopping therapy, perhaps up to 10 times ULN, may be more appropriate. The optimal order and method of drug re-introduction is unknown, and no randomised comparisons have ever been published within the context of TBM treatment.

Dexamethasone induced adrenal suppression

Iatrogenic administration of exogenous corticosteroids is associated with adrenal suppression, and the sudden cessation of treatment can lead to Addisonian crises which can be life-threatening. For this reason it is common practice to prescribe corticosteroids in tapering doses, to allow recovery of the adrenal cortex during the treatment period. Thus, by the time the corticosteroid is finished, normal endogenous cortisol production will have resumed with no risk of Addisonian crisis. Whether the use of corticosteroids in the doses prescribed in infectious diseases are associated with significant adrenal suppression is not clear and has not been investigated.

HIV-associated TBM

Co-treatment of HIV and tuberculosis

HIV infection increases the risk of an individual infected with *M. tuberculosis* developing TBM, complicates treatment, and substantially worsens outcome^{10–12}. Co-treatment of HIV and tuberculosis is complex because of the adherence demands of multidrug therapy for two infections, drug-drug interactions between rifampicin and ART, overlapping side effect profiles of anti-tuberculosis drugs and ART, and the frequency of IRIS. The optimal strategy for starting ART in TBM is unclear.

Use of adjunctive dexamethasone

How corticosteroids confer a survival benefit, and whether they do so in HIV-infected patients with TBM, remains uncertain. In TBM dexamethasone may reduce the early intracerebral inflammatory response, prevent hydrocephalus infarction and tuberculoma formation, and reduce the incidence of neurological IRIS. Dexamethasone may reduce the risk of DILI and thereby improve outcome by enabling uninterrupted anti-tuberculosis treatment. The current evidence-base for using adjunctive corticosteroids for the treatment of HIV-associated TBM is restricted to 98 adults recruited to a trial in Vietnam¹³. This trial randomised a total of 545 subjects (98 of them HIV-positive) and reported an overall reduction in 9-month mortality due to dexamethasone from 41.3% (112/271) to 31.8% (87/274) (hazard ratio of time to death 0.69; 95% confidence interval (CI) 0.52–0.92, $P=0.01$).

While there was no clear evidence of treatment effect heterogeneity according to HIV status, the number of included HIV-infected subjects was low and the observed benefit in that subgroup was smaller: 61.4% (27/44) in the dexamethasone group died, compared to 68.5% (37/54) in the placebo group (hazard ratio of time to death 0.86; 95% CI 0.52–1.41; $P=0.55$). On the basis of these data most international guidelines cautiously recommend dexamethasone should be given for HIV-associated TBM, but all acknowledge the paucity of evidence and the need for additional controlled trial data.

Study hypotheses

Neurological complications are both common and devastating in TBM. Dexamethasone may reduce complications arising from an early intracerebral inflammatory response, including neurological IRIS. Dexamethasone has been shown to improve survival in HIV-unaffected individuals with TBM. **Our primary hypothesis is that adjunctive dexamethasone increases survival from TBM in HIV co-infected adults.** The secondary hypothesis is that current guidelines for the management of anti-tuberculosis DILI in those with TBM result in the premature interruption of rifampicin and isoniazid (the critical active drugs in early therapy) and are thereby placing participants at risk of poor outcomes.

Study aims

Primary aim

Our primary aim is to determine whether dexamethasone is a safe and effective addition to the first 6–8 weeks of anti-tuberculosis treatment of TBM in patients with HIV with dexamethasone duration depending on Medical Research Council (MRC) grade (Supplementary File 1) at the start of treatment. In making this assessment we not only determine whether dexamethasone improves survival, but also whether it lengthens the time to new neurological events, IRIS, drug-related adverse events, opportunistic infections, and disability assessed by the modified Rankin scale (Table 1). We will follow participants for 24 months to assess longer-term neurological outcomes and the incidence of HIV-associated malignancy in the two treatment arms.

Secondary aim

Our secondary aim is to investigate alternative management strategies in a subset of patients who develop DILI that will enable the safe continuation of rifampicin and isoniazid therapy whenever possible.

Design and setting

ACT HIV is a parallel group, randomised (1:1), double blind, placebo-controlled multi-centre Phase III trial, comparing dexamethasone versus placebo for 6–8 weeks in addition to standard anti-tuberculosis and antiretroviral treatment. The trial will be set in two hospitals in Ho Chi Minh City, Vietnam and two hospitals in Jakarta, Indonesia. The trial will enrol 520 HIV-infected adults (≥ 18 years old) admitted to participating hospitals with a suspected diagnosis of TBM, as judged by the attending physician, and requiring immediate anti-tuberculosis treatment. Doctors making the diagnosis of TBM will all be senior physicians specialising in either infectious diseases or tuberculosis and lung diseases. All will receive additional diagnostic training and follow a diagnostic standard operating procedure. This trial schema is shown in Figure 1.

An ancillary DILI strategy study will perform a randomised comparison of management strategies in DILI early in anti-tuberculosis treatment (the intensive phase). We will perform an open, randomised comparison of three management strategies with the aim of demonstrating which strategy results in the least interruption in rifampicin and isoniazid treatment. All patients enrolled in the main trial will be eligible to take part in this study, with the exception of those known to have TBM caused by isoniazid resistant or MDR *M. tuberculosis*.

Ancillary studies

Seven ancillary studies will be conducted within the ACT HIV trial. Some of the studies will only involve a subset of patients recruited at the Hospital for Tropical Diseases (HTD), Ho Chi Minh City, Vietnam. Ancillary studies 2, 3 and 7 will be retrospective data analyses. Ancillary study 4 and 5 will collect and review patient results as the trial progresses. For ancillary study 6 results will be fully analysed at the end of the trial, however laboratory values that may influence clinical care will be available to the clinical team as the trial progresses.

The studies are as follows:

Ancillary study 1: A randomised comparison of management strategies in response to DILI (as above). (All patients.)

Ancillary study 2: Host and bacterial genetic determinants of treatment response. We hypothesise that the LTA4H gene, and genes involved in related inflammatory pathways, may additionally influence participant inflammatory state,

Table 1. Modified Rankin scale.

Score	Description
0	No symptoms
1	Minor symptoms not interfering with lifestyle
2	Symptoms that lead to some restriction in lifestyle, but do not interfere with the patients ability to look after themselves
3	Symptoms that restrict lifestyle and prevent totally independent living
4	Symptoms that clearly prevent independent living, although the patient does not need constant care and attention.
5	Totally dependent, requiring constant help day and night.
6	Death

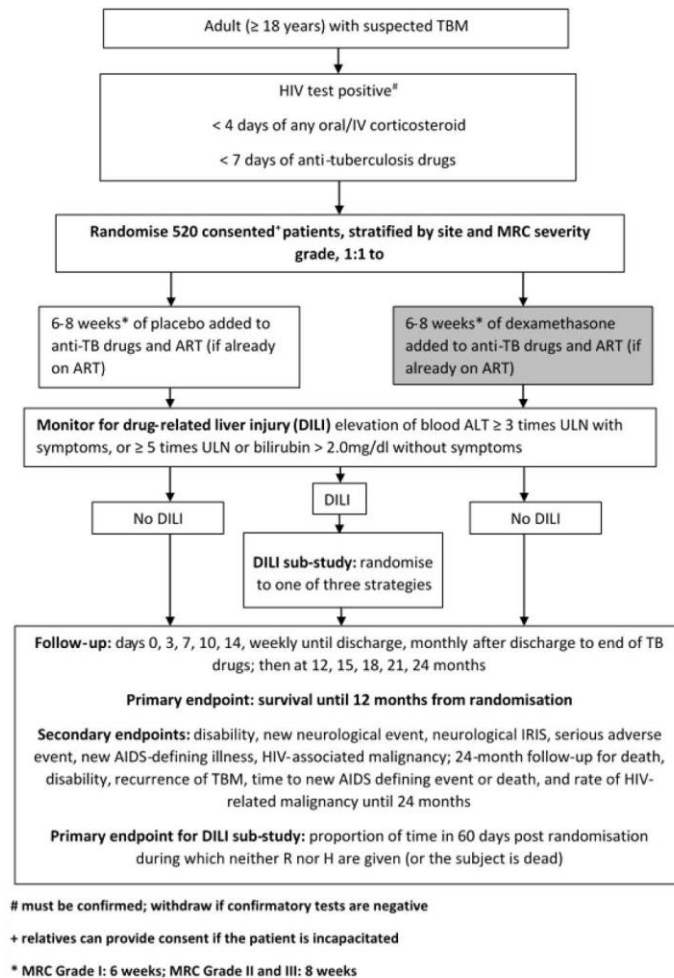


Figure 1. Trial Schema (main trial).

TBM severity, and treatment response. We will also examine genetic variants associated with the development of DILI. (All patients.)

Ancillary study 3: Impact of dexamethasone on CSF inflammation and gross cerebral pathology (assessed by serial brain magnetic resonance imaging (MRI)). We will investigate how dexamethasone influences the

resolution of inflammatory markers in the CSF and the gross pathological consequences of TBM on the brain (hydrocephalus, stroke, and tuberculoma formation). (HTD patients only.)

Ancillary study 4: Influence of diabetes mellitus on presentation and response to treatment. We will investigate whether diabetes mellitus influences clinical presentation

and CSF inflammatory phenotype (linking with ancillary study 2), and how it impacts upon treatment outcomes. (All patients.)

Ancillary study 5: Influence of *Strongyloides* infection on presentation and response to treatment. We will determine whether *Strongyloides* co-infection alters clinical and/or CSF inflammatory phenotype at TBM presentation (linking with ancillary study 2) and treatment response. (All patients.)

Ancillary study 6: Pathophysiology and treatment of hyponatraemia and raised intracranial pressure. We will investigate the pathophysiology of TBM-associated hyponatraemia, enabling a better understanding of the causes of hyponatraemia, the relationship between plasma sodium and elevated intracranial pressure, and the best management of severe hyponatraemia. (HTD patients only.)

Ancillary study 7: Dexamethasone induced adrenal suppression. We will investigate whether the use of corticosteroids in the doses prescribed in infectious diseases are associated with significant adrenal suppression. (HTD patients only.)

Endpoints

Primary endpoint – ACT HIV (main trial)

The primary endpoint of the main trial is overall survival, i.e. the time from randomisation to death, during a follow-up period of 12 months. Survivors known to be alive at 12 months will be censored at that time-point and subjects who withdrew or were lost to follow-up before 12 months will be censored at the date they were last known to be alive.

Primary endpoint – DILI strategy study

In the DILI strategy study the primary endpoint is the proportion of time in the 60 days following randomisation during which neither rifampicin nor isoniazid are given (or the subject is dead).

Secondary endpoints – ACT HIV (main trial)

The secondary endpoints of the main trial are as follows:

- Neurological disability at day 30 from randomisation, monthly until completion of anti-tuberculous drugs, and at months 12, 18 and 24. The main endpoint is the 12-month assessment and subjects who died before 12 months will be treated as having a score of 6 ('dead'). Neurological disability will be assessed by the modified Rankin score (Table 1).
- Time to first new neurological event or death during a follow-up period of 12 months. A neurological event is defined as a fall in Glasgow Coma Score (GCS) by ≥ 2 points for ≥ 2 days from the highest previously recorded GCS (including baseline), or the onset of any of the following clinical adverse events: cerebellar symptoms, focal neurological signs, or seizures.
- Time to neurological IRIS events from randomisation until 6 months. We will follow the International Network for the Study of HIV associated IRIS (INSIH) case

definition of IRIS (14, Supplementary File 2). Rate of neurological IRIS is defined as the number of IRIS events divided by the observed person-time of follow-up in each treatment group.

- Time to new acquired immunodeficiency syndrome (AIDS)-defining event (as per World Health Organisation classification) or death, during a follow up period of 12 months.
- Time to HIV-associated malignancy from randomisation until 12 months. Rate of HIV-associated malignancy is defined as the number of events of the three major HIV-associated malignancies (Kaposi sarcoma, high grade B-cell non-Hodgkin lymphoma and invasive cervical cancer) divided by the observed person-time of follow-up in each treatment group.
- Serious adverse events until 12 months. Comparison of the frequency of serious adverse events between treatment groups will form an important part of the study analysis.
- Endpoints assessed at 24 months of follow up. All participants will continue to be followed up for 24 months and the following outcomes will be reported once this period has been completed for all participants: overall survival until 24 months, neurological disability at 24 months, recurrence of TBM until 24 months, time to new AIDS defining event or death until 24 months, and rate of HIV-related malignancy until 24 months.

Secondary endpoints – DILI strategy study

The secondary endpoints of the DILI strategy study are as follows:

- Development of acute liver failure (defined as new onset coagulopathy (International normalised ratio (INR) >1.5) and hepatic encephalopathy) after randomisation.
- ART interrupted due to drug-related injury.
- Time to new neurological event (defined as a fall in GCS of ≥ 2 points for ≥ 48 hours, new focal neurological sign, or new onset of seizures) or death from randomisation until the 12 month follow-up of the main trial.
- Overall survival, i.e. time to death from any cause, until the 12 month follow-up of the main trial.
- Neurological disability at the 12 month follow-up of the main trial.

Inclusion and exclusion criteria

Inclusion criteria

Study participants for ACT HIV must be adults (aged 18 years or older), HIV-infected, with a clinical diagnosis of TBM (≥ 5 days of meningitis symptoms, and CSF abnormalities) and anti-tuberculosis chemotherapy either planned or started by the attending physician. Participants will be considered eligible for enrolment in this trial if they fulfil all the inclusion criteria and none of the exclusion criteria. TBM is a serious infection whose standard treatment requires hospitalisation. Therefore all

eligible participants will be treated in hospital, at least for the initial 3 weeks of their illness. Pregnant participants are eligible for enrolment. Participants with drug resistant TBM are an important sub group, and are eligible for enrolment into the main study (but not the DILI ancillary study).

Study participants for the DILI strategy study must be receiving first-line anti-tuberculosis drugs and fulfil the definition of drug-related liver injury: elevation of blood transaminase concentrations ≥ 3 times the ULN with symptoms and signs of hepatitis (vomiting, abdominal pain, jaundice), or ≥ 5 times the ULN or a rise in serum bilirubin $> 2.0 \text{ mg/dL}$ ($> 34 \text{ } \mu\text{mol/L}$) without symptoms⁸, and less than 90 days of anti-tuberculosis drugs given.

Exclusion criteria

Exclusion criteria for ACT HIV are:

- An additional brain infection (other than TBM) confirmed or suspected: positive CSF Gram or India Ink stain; positive blood or CSF Cryptococcal antigen test; cerebral toxoplasmosis suspected and attending physician wants to give anti-toxoplasmosis treatment with anti-tuberculosis treatment.
- More than 6 consecutive days of two or more drugs active against *M. tuberculosis* immediately before screening.
- More than 3 consecutive days of any type of orally or intravenously administered corticosteroid immediately before randomisation.
- Dexamethasone considered mandatory for any reason by the attending physician.
- Dexamethasone considered to be contraindicated for any reason by the attending physician.
- Patient has previously been randomised into this trial for a prior episode of TBM.
- Lack of consent from the participant or family member (if the participant is incapacitated by the disease).

Exclusion criteria for the DILI strategy study are:

- TBM known to be caused by isoniazid resistant or MDR *M. tuberculosis* or standard first-line anti-tuberculosis drugs unable to be given for any reason other than DILI.
- Signs of chronic liver disease of any cause (hepatosplenomegaly, prolonged jaundice, caput medusae, spider angiomas, ascites, oedema).
- Lack of consent from the participant or family member (if the participant is incapacitated by the disease).
- Elevation of blood transaminase concentrations ≥ 3 times the ULN with symptoms and signs of hepatitis (vomiting, abdominal pain, jaundice), or ≥ 5 times the ULN or a rise in serum bilirubin $> 2.0 \text{ mg/dL}$ ($> 34 \text{ } \mu\text{mol/L}$) without symptoms at baseline (day 0).

Recruitment, retention and randomisation

Recruitment and retention

Recruitment activities will only occur in an inpatient hospital setting in participating hospitals. The target sample size of 520 participants will be enrolled into the main trial, with an anticipated accrual rate of 4 years. Once discharged from hospital the participants will be contacted by phone to remind them of their next visit. In addition, patients who have missed a visit will be contacted by phone for a maximum of three times after which a maximum of three home visits can be conducted. All contact attempts will be recorded. As part of routine clinical care participants with suspected TBM will have an HIV test, a lumbar puncture, and a GeneXpert MTB/RIF test on CSF to assess the likelihood of *M. tuberculosis* infection and rifampicin resistance. When possible, participants will be screened for eligibility on the day their CSF results return and at the time the decision is made to start anti-tuberculosis chemotherapy for suspected/confirmed TBM. The name and date of birth of every adult screened for the trial should be added to the site Screening and Randomisation Register, together with the allocated trial number if subsequently randomised, or the reason the participant was not randomised. We anticipate 100 ACT-HIV patients will consent to the ancillary DILI strategy study.

Randomisation

Randomisation to ACT HIV will be stratified by participating hospital/site and modified MRC TBM severity grade. The randomisation list will be computer-generated based on random permuted blocks with variable block size following Oxford University Clinical Research Unit (OUCRU) standard operating procedures. A 24h web-based randomisation service will be provided. The OUCRU biostatistician in charge of randomisation list preparation will set up statistical code to generate the randomisation list and transfer it to the Study Pharmacist. The Study Pharmacist will then change the random seed, i.e. the initialisation of the random numbers generator, in the statistical code in order to blind the biostatistician and then run the code to prepare the final randomisation list. The generated randomisation lists will be securely incorporated within the trial database. A reliable manual back-up system will also be available. Randomisation to the three strategies for DILI will be 1:1:1 with stratification by initial randomisation (dexamethasone or placebo) and site. Authorised individuals will receive an allocated treatment strategy by visiting the same website used for the primary randomisation.

Blinding, unblinding and treatment discontinuation

Blinding

All participants and investigators will be blinded to the treatment allocation. OUCRU clinical trials unit (CTU) pharmacists will create blinded drug packages (in fully made-up and labelled treatment packs) containing either active drugs or identical placebo sufficient for 6–8 weeks of treatment (dependent on the MRC grade of the participant) according to the prespecified randomisation list, and pre-ship them to the sites. After randomisation, the ward or trial research nurse will take the completed prescription form to the site pharmacy; they will dispense the

trial-number specific pack containing the study drug. Unused drug will be returned to the site pharmacy if a participant withdraws from treatment. In ancillary study 3 MRI brain images will be read by an independent neuroradiologist, blind to the treatment allocation and outcomes of the participant.

Unblinding

If, in the opinion of the local clinician, it is important for good clinical care to unblind treatment the documented request will be discussed with the site Principal Investigator (PI) and Chief Investigator (CI). If it is agreed that knowledge of treatment allocation is essential for the best management of the patient, the unblinding code will be provided by the study pharmacist holding the randomisation list at OUCRU CTU upon documented request from the CI. Generalised clinical deterioration is not sufficient for unblinding, given equipoise about the evidence base supporting the use of dexamethasone regardless of clinical severity. All instances of unblinding will be recorded and reported to the Data Monitoring Committee (DMC) and Trial Steering Committee (TSC).

Protocol treatment discontinuation

An individual participant may stop study drug early for any of the following reasons: Participant no longer believed to have TBM and all anti-tuberculosis treatment stopped; Confirmatory HIV tests are negative and HIV infection excluded; Unacceptable toxicity or adverse event; Intercurrent illness that prevents further treatment; Any change in the participant's condition that justifies the discontinuation of treatment in the treating physician's opinion and after discussion with the site PI; Inadequate compliance with the protocol treatment in the judgement of the treating physician; Withdrawal of consent for treatment by the participant.

Trial management

Interventions

All participants will receive the standard of care anti-TB drugs and ART drugs according to respective national guidelines. In ART-naïve patients, ARTs will be started 6–8 weeks after the start of anti-tuberculosis drugs. Study participants for the main trial will be randomised to receive either dexamethasone

or placebo (investigational medicinal product (IMP)) as an extra medication for 6–8 weeks, dependent upon the severity of TBM disease (Table 2). Dexamethasone/placebo will be dispensed at randomisation from the site pharmacy in intravenous and oral (tablet) formulations. Placebo will be identical in appearance to active drug and dosed and dispensed in the same way. The study drug will be given to participants as early as possible in the treatment of TBM but no later than 7 days from the start of anti-tuberculosis treatment.

Participants for the DILI strategy study will be randomised to one of three strategies (Figure 2): Strategies are as follows:

- 1) Observe: measure transaminases, bilirubin, and INR every 3 days; do not change/stop anti-tuberculosis drugs unless transaminases rise to $\geq 10\times$ normal, or total bilirubin rises $>2.5\text{mg/dl}$ ($>43\text{ }\mu\text{mol/L}$), or INR >1.5 or symptoms of hepatitis worsen (nausea, vomiting, abdominal pain), in which case go to Strategy 3.
- 2) Stop pyrazinamide (Z) alone. Observe, measuring transaminases, bilirubin, and INR every 3 days. If transaminases do not fall to $<5\times$ ULN by day 5, or total bilirubin rises $>2.5\text{mg/dl}$ ($>43\text{ }\mu\text{mol/L}$), or INR >1.5 or symptoms of hepatitis worsen at any time (nausea, vomiting, abdominal pain), go to Strategy 3.
- 3) Current standard of care (current US CDC guidelines⁴, Supplementary File 3).

Patients in the DILI study will undergo regular clinical and blood test monitoring, and there is a clear procedure to follow for moving patients from strategy 1 to strategy 3, or from strategy 2 to strategy 3. We hypothesise that current guidelines for the management of anti-tuberculosis DILI result in the premature interruption of rifampicin and isoniazid (the critical active drugs in early therapy) in those with TBM and are thereby placing participants at risk of poor outcomes. Strategies 1 and 2 may demonstrate improved safety compared to strategy 3. However, regular clinical monitoring, and interim review by an independent data monitoring committee, will be in place to ensure safety of participants in case of any increased adverse events in any of the DILI strategy groups.

Table 2. Study drug treatment regimen following randomisation.

	MRC Grade I Daily dexamethasone dose/route	MRC Grades II and III Daily dexamethasone dose/route
Week 1	0.3 mg/kg/24 hrs IV	0.4 mg/kg/24 hrs IV
Week 2	0.2 mg/kg/24 hrs IV	0.3 mg/kg/24 hrs IV
Week 3	0.1 mg/kg/24 hrs IV	0.2 mg/kg/24 hrs IV
Week 4	3mg/24 hrs oral	0.1 mg/kg/24 hrs IV
Week 5	2mg/24 hrs oral	4 mg/24 hrs oral
Week 6	1 mg/24 hrs oral	3 mg/24 hrs oral
Week 7	Stop	2 mg/24 hrs oral
Week 8		1 mg/24 hrs oral

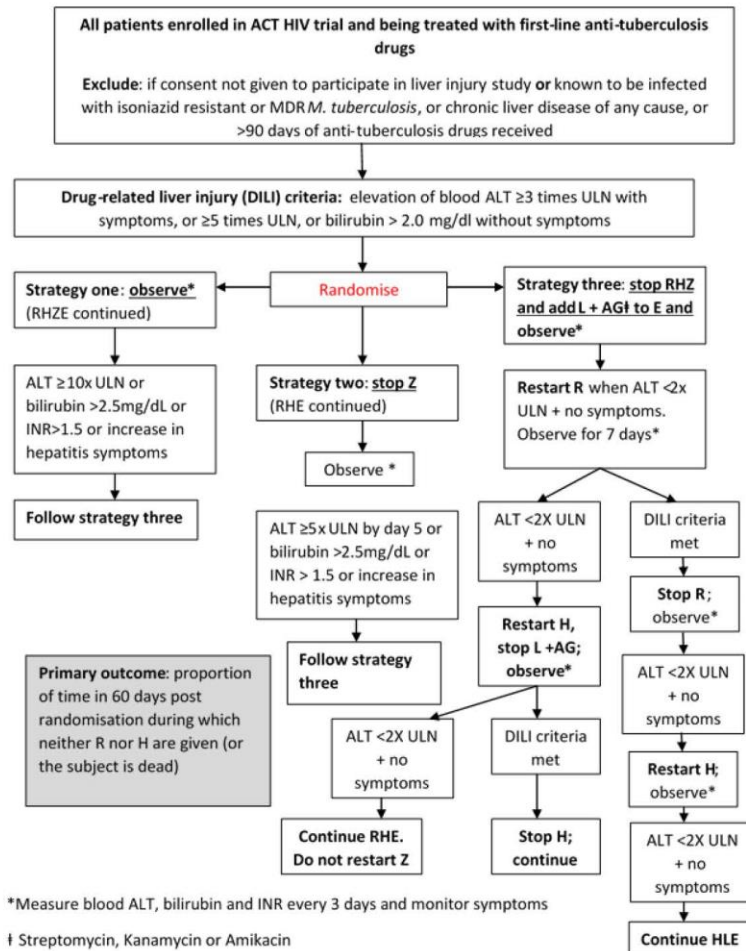


Figure 2. Trial Schema (drug-induced liver injury strategy study).

DILI related interventions are conducted within the ACT HIV trial, but the DILI strategies (specifically arms 1 and 2, compared with the routine care arm 3) are not expected to increase adverse events. It is in fact anticipated that arm 1 (continue all 4 first line anti-TB drugs) and arm 2 (stop only pyrazinamide) of the DILI study will result in less adverse events due to the continuation of the best and most appropriate anti-TB drugs. Liver function

tests will be closely monitored. DILI occurs more frequently in individuals with HIV therefore it is very important that DILI interventions are assessed in an HIV positive group, to avoid unnecessary cessation of first line anti-TB drugs if arms 1 and / or 2 are effective. From a safety perspective, if a patient develops DILI, prior randomisation to dexamethasone or placebo should not affect DILI in an adverse way.

Anti-tuberculosis treatment

First line anti-tuberculosis treatment will follow current Vietnamese and Indonesian national guidelines. Rifampicin (10mg/kg/24 hrs; maximum 600mg), isoniazid (5mg/kg/24hrs; maximum 300mg), pyrazinamide (25mg/kg/24hrs; maximum 2g) and ethambutol (20mg/kg/24 hrs; maximum 1.2g) will be given for at least the first 2 months of treatment, provided drug resistance is not suspected or proven. Pyrazinamide will then be stopped and rifampicin, isoniazid and ethambutol (at the same doses) will then be given until at least 12 months anti-tuberculosis treatment in total has been given. Patients with visual complications will discontinue ethambutol and an alternative drug will be used in its place. The decision of which fourth drug to use will be made by the treating physician, and is not mandated by the trial. In practice this will be either a fluoroquinolone or streptomycin.

For TBM caused by isoniazid-resistant tuberculosis the attending physician should decide which option to take, dependent upon clinical circumstances. For participants with MDR tuberculosis, second-line treatment should be given as soon as possible, following national guidelines and local policies.

Participants with abnormal liver function tests (LFTs) at screening are eligible to enter the main trial. These patients should be given standard first-line anti-tuberculosis treatment unless blood transaminase concentrations are ≥ 3 times the ULN with symptoms and signs of hepatitis (vomiting, abdominal pain, jaundice), or ≥ 5 times the ULN or a rise in serum bilirubin $>2.0\text{mg/dL}$ ($>34\mu\text{mol/L}$) without symptoms. In these participants, initial anti-tuberculosis treatment should consist of levofloxacin, ethambutol, and an aminoglycoside (either kanamycin, amikacin or streptomycin). LFTs should be monitored every 3 days and rifampicin started as soon as blood transaminases are $<5\times$ the ULN and/or the symptoms and signs of hepatitis resolve. Once the participant is tolerating a rifampicin-containing regimen isoniazid can be introduced and, if isoniazid is tolerated, the aminoglycoside can be stopped. If pyrazinamide is not used in treatment, at least 12 months of anti-tuberculosis treatment must be given. Participants with liver dysfunction at the start of treatment that require modified initial anti-tuberculosis treatment regimens are ineligible for the DILI strategy study.

Anti-retroviral therapy

ART will be provided for all participants within the current Vietnamese and Indonesian national guidelines. ART in both Ho Chi Minh City and Jakarta is often commenced 6–8 weeks after the start of anti-tuberculosis treatment, with the precise timing left to the discretion of the attending physician. Delays in starting ART of longer than 8 weeks are not encouraged. This practice is supported by our previous trial of immediate versus deferred ART in TBM and consistent with current local practice guidelines¹⁵. The optimal time to start ART in TBM is not known, depends upon TBM disease severity and CD4 count, and varies between centres regarding routine practice. Exactly when to start ART is decided upon by the treating physician. A patient with known HIV already on ART will continue this ART when they enter the trial, and during the trial. Whenever possible, patients

will be treated with an efavirenz-containing regimen. When viral resistance to first line (NNRTI-based) ART is suspected, case-by-case decisions will be made with regard to HIV-RNA measurement, drug resistance testing, and composition and timing of second line HIV treatment and concurrent tuberculosis treatment, avoiding combined use of rifampicin and protease inhibitors because of strong drug interactions and high risk of toxicity¹⁶. Decisions on dose or schedule adjustments for these participants will be made on an individual basis, following the national and local guidelines. If DILI develops ART should not be stopped until all other management has been tried (including interrupting anti-tuberculosis drugs according to the randomised schedule). HIV specialists and liver specialists should be consulted at this point.

Prophylaxis for opportunistic infections

All participants will receive *Pneumocystis jirovecii* prophylaxis with co-trimoxazole according to national guidelines (CD4 count <200 cells/mm³). In the DILI strategy study prophylactic co-trimoxazole and/or fluconazole should be stopped before all other drugs if DILI develops.

Use of concomitant medication

All other concomitant medications essential for participant management are permitted at enrolment, subject to the exclusion criteria of no contraindications to the use of dexamethasone in the judgement of the attending clinician. If use of a concomitant medication that cannot safely be used with dexamethasone becomes essential after randomisation, then the IMP should be stopped and the concomitant medication used without unblinding. Drugs which increase the risk of gastrointestinal bleeding, such as non-steroidal anti-inflammatory drugs (NSAIDs), should be used with caution. Any other oral or intravenously administered corticosteroids are not permitted, unless deemed essential, in which case the study drug should be stopped and replaced by the chosen corticosteroid. The treatment of tuberculosis and HIV once the study drug has been stopped should be determined by national guidelines and the physician responsible for the care of the participant.

Management of neurological complications occurring after the start of anti-tuberculosis treatment

Neurological deterioration, manifest by falling conscious level or new focal neurological signs, is common after the start of treatment of TBM². Common causes are hydrocephalus, stroke, tuberculomas, and hyponatraemia, and neurological events occurring within 2 months of starting ART may also be due to IRIS. Whenever possible, all patients with unexplained neurological deterioration should have either brain imaging with computed tomography (CT) or MRI. Corticosteroids are not routinely recommended for the treatment of hydrocephalus or stroke. Tuberculomas may cause significant peri-lesional inflammation and oedema and can manifest as new onset seizures, focal neurological deficit, or globally reduced consciousness. In these circumstances, most physicians recommend using corticosteroids. Therefore, if after clinical and neuroradiological review the attending physician believes a patient's neurological deterioration is due to tuberculoma(s), open-label dexamethasone is

recommended. Study drug should be stopped, if it still being given (without the need for unblinding), and high-dose intravenous dexamethasone (0.4mg/kg/24hrs) should be prescribed. The speed of dexamethasone reduction and the total duration of therapy should be determined on a case-by-case basis by the attending physician.

Management of IRIS

Untreated HIV-infected patients starting ART treatment for the first time after at least 2 weeks of anti-tuberculosis treatment will be at risk of developing neurological IRIS. A single randomised controlled trial demonstrated that corticosteroids reduced the need for hospitalisation and therapeutic procedures and hastened improvements in symptoms, performance, and quality of life in IRIS associated with non-neurological tuberculosis IRIS¹⁷. Therefore, in the absence of other data, if the criteria for IRIS are met, then we recommend the participant should be given open-label dexamethasone or prednisolone. If the participant has IRIS with neurological involvement then intravenous dexamethasone 0.4mg/kg/24 hrs should be given. Oral prednisolone (following the published trial regimen¹⁷) should be given for non-neurological IRIS (prednisone 1.5mg/kg per day for 2 weeks, then 0.75mg/kg per day for 2 weeks). IRIS will be diagnosed if the participant meets the antecedent requirements, clinical IRIS criteria, and alternative explanations for the clinical deterioration are excluded if possible ([Supplementary File 2](#)). Cases where alternative diagnoses cannot be fully excluded because of limited diagnostic capacity should be regarded as "probable paradoxical tuberculosis-associated IRIS". In these probable cases, should resolution of clinical or radiological findings of the suspected IRIS episode occur without a change in tuberculosis treatment or ART having been made, they could then be reclassified as "paradoxical tuberculosis-associated IRIS" cases.

Data collection

The trial assessment schedule for ACT HIV is outlined in [Table 3](#).

Clinical assessment

Clinical assessment will include conscious level by GCS, new or ongoing focal neurological deficit, clinical treatment response, all serious adverse events including new malignancies and opportunistic infections, all adverse events of any grade leading to modification of anti-tuberculosis treatment or ART or their interruption/early discontinuation (and clinician-assessed likelihood of relationship of adverse event to dexamethasone), and adherence to drugs (study drug, anti-tuberculosis drugs and ART). Assessment of disability by the modified Rankin score will be performed at day 30 from randomisation, monthly until completion of anti-TB drugs, and at months 12, 18 and 24.

Inpatient assessment

The clinical team will record daily GCS and new focal neurology throughout the participant's hospital admission. Participants will be visited by one of the research team at screening, baseline, and at least every 3 days for the first 4 weeks of treatment (unless they are discharged or die before 4 weeks) and then at least every 7 days whilst they remain in hospital.

Formal trial clinical assessments will occur on day 0, 3, 7, 10, 14, and weekly thereafter until discharge (+/- 1 day).

Outpatient assessment

After discharge clinical assessments will occur monthly until 12 months. Some of these assessments can be made by phone. Formal outpatient review will occur monthly (+/- 7 days) for at least the first 2 months following hospital discharge. The patient should have formal outpatient review at least every 2 months until month 12 after randomisation. Thereafter patients should be followed up by phone call at 15 months and 21 months and by formal outpatient review at 18 months and 24 months from randomisation.

HIV monitoring

HIV infection must be confirmed before entry to the study. Peripheral blood CD4 count and HIV viral load will be measured at baseline (routine counts taken within 2 weeks of study entry are acceptable), and CD4 count will be repeated at 60 days.

Liver function

Alanine transaminase (ALT) and bilirubin will be measured to evaluate liver toxicity every 7 days until discharge.

Additional blood tests

EDTA blood will be taken for hepatitis B surface antigen and hepatitis C antibodies, and serum will be stored for later DNA extraction where consent has been given. We will use the DNA to investigate novel genetic determinants of treatment response, including LTA4H genotype, and the development of DILI.

Glycosylated haemoglobin (HbA1c) and fasting blood sugar

HbA1c and fasting blood sugar will be measured at baseline and at 60 days from randomisation. This will enable determination of the frequency of undiagnosed diabetes in those presenting with TBM, assess diabetic control in those known to have diabetes, and evaluate the influence of dexamethasone and anti-tuberculosis treatment on diabetic control over the first 60 days of TBM treatment. To enable more detailed phenotyping of diabetes we will also measure C-peptide and blood lipids at baseline and store plasma for future diabetes related auto-antibody testing.

Strongyloides

All enrolled participants will be tested for serological evidence of Strongyloides infection (past or latent infection) with stool examination for evidence of active infection at baseline and at 60 days from randomisation. When infection is detected, treatment with ivermectin will be provided. High-dose corticosteroids can lead to re-activation of latent Strongyloides and hyperinfection syndrome. Stool will be examined for Strongyloides larvae on day 60 (end of study drug) to determine whether reactivation alters TBM treatment responses.

Synacthen test

We will compare adrenal responsiveness at 3 weeks after randomisation and at the end of study drug treatment (6 or

Table 3. ACT HIV trial assessment schedule.

	SCREENING	DAYS				MONTHS						
		0	3	7	WEEKLY UNTIL DISCHARGE	30	60	MONTHLY TO ANTI-TB DRUG END	12	18	24	
ALL PARTICIPANTS												
Eligibility assessment	(X)											
Participant information sheet and consent	X											
Randomisation		X										
Clinical assessment	(X)	X	X	X	X	X	X	X	X	X	X	
Disability assessment						X	X	X	X	X	X	X
Chest X-ray	(X)						X			X		
Lumbar puncture (with paired plasma glucose)	(X)					X	X					
CSF Cryptococcal Ag test	(X)											
HIV test	(X)											
EDTA blood for genetic tests		X										
Full blood count	(X)			(X)								
CD4 count	(X)						X					
HIV viral load		X										
Storage for later DNA extraction		X										
Sodium	(X)				(X)			(X)				
Urea/Creatinine	(X)				(X)							
ALT/ bilirubin	(X)			(X)	(X)			(X)				
Hepatitis C antibodies		X										
Hepatitis B surface antigen		X										
Fasting blood sugar/ HbA1c		X					X					
C-peptide/Lipids		X										
Strongyloides serology		X										
Serum Storage		X										
Stool for Ova, cyste and parasites (Strongyloides) microscopy		X					X					
SUBSET OF PARTICIPANTS RECRUITED TO IMAGING STUDY (HTD Vietnam only)												
Brain MRI		X					X			X		
SUBSET OF PARTICIPANTS RECRUITED TO HYPONATRAEMIA/ICP ANCILLARY STUDY (HTD Vietnam only)												
24-hour fluid balance	(X)	X	X	X	X							
Plasma sodium		X	X	X	X							
Plasma osmolality												
Urinary sodium		X	X	X	X							
Urinary osmolality												
Plasma cortisol		X										
Ultra-sound assessment of intravascular volume		X	X	X	X							
Ultra-sound measurement of optic nerve sheath diameter		X	X	X	X							
SUBSET OF PARTICIPANTS RECRUITED TO ADRENAL SUPPRESSION ANCILLARY STUDY (HTD Vietnam only)												
Synacthen test					X (day 21)		X					

8 weeks depending upon disease severity) in 100 consecutive patients using the short Synacthen test. The patient's background cortisol level is measured by drawing 2mls of blood at 0900hrs. 250mcg of Synacthen is then administered intravenously; 3ml samples of blood are taken at thirty minutes and sixty minutes to measure the cortisol level after this adrenal stimulation. Synacthen test will be repeated at day 60 after randomisation.

Lumbar puncture

Lumbar puncture should be performed, unless clinically contraindicated, as part of routine clinical care for the baseline assessment and on days 30 and 60 after randomisation to assess treatment response. Day 60 lumbar punctures are not routine practice in the Jakarta hospitals and will therefore not be mandated by the trial in these centres. Opening pressure should be measured, at least 5mls of CSF should be taken for mycobacterial investigations alone, and assessments of cell count and differential, protein, glucose (paired with serum), and lactate should also be performed on 1–2 mls of additional CSF. Ziehl Neelsen (ZN) stain, GeneXpert, and *M. tuberculosis* culture will be performed on all CSF taken. As part of an ancillary study of the impact of dexamethasone on CSF inflammation and gross cerebral pathology we will measure concentrations of a variety of inflammatory mediators in the CSF (leucocytes, cytokines, chemokines, and eicosanoids, for example) at baseline and on days 30 and 60 after randomisation to determine how dexamethasone influences their expression.

Hypnatraemia and raised intracranial pressure

In the subset of participants enrolled to HTD, Ho Chi Minh City, Vietnam, we will investigate the pathophysiology of TBM-associated hyponatraemia by serial assessments of fluid balance, paired plasma and urinary sodium and osmolality, and intravascular volume by Doppler ultrasound assessment of inferior vena cava collapsibility index^{18,19}. We will also use portable ultrasound to measure the optic nerve sheath diameter, which has been shown to be a reliable and non-invasive measure of raised intracranial pressure^{20,21}. These additional measurements will be assessed at days 0, 3, and 7, and weekly until discharge. Plasma cortisol will be measured at day 0.

Imaging

Chest Xray will be performed at screening and on day 60 and 12 months after randomisation. In the subset of participants enrolled to HTD, Ho Chi Minh City, Vietnam, we will perform brain imaging by MRI (or CT if the participant cannot tolerate an MRI) at baseline (+/- 7 days) and at 60 days (+/- 7 days) and at 12 months (-0/+1 month). At sites other than HTD, Ho Chi Minh City, Vietnam, brain imaging is performed based upon the routine care at that site. Brain imaging may not always be readily available, and this study does not mandate brain imaging for every patient. Brain imaging for suspected TBM is strongly supported, and individual decisions will be made by the treating physician.

Adverse events and safety reporting

Adverse events

Specific procedures will be followed when notifying and reporting adverse events (AEs) or adverse reactions (ARs). The

definitions of the EU Directive 2001/20/EC Article 2 based on the principles of ICH good clinical practice (GCP) apply to this trial protocol. All AEs and ARs will be assessed as to whether they are serious or not. If the event is serious and not only related to TBM, or is fatal, then a serious adverse event (SAE) form must be completed and the OUCRU CTU notified within 24 hours. All AEs and ARs (serious and non-serious) should be graded using toxicity gradings.

Causality of all SAEs or serious adverse reactions (SARs) in relation to the trial therapy (dexamethasone) will be assessed. There are five categories: unrelated, unlikely, possible, probable, and definitely, related. If the causality assessment is unrelated or unlikely to be related, the event is classified as an SAE. If the causality is assessed as possible, probable or definitely related, then the event is classified as an SAR. If there is at least a possible involvement of the trial treatment (or comparator), the investigator must assess the expectedness of the event. An unexpected adverse reaction is one not previously reported in the current Summary of Product Characteristics (SPC) at the time the event occurred, or one that is more frequent or more severe than previously reported. If a SAR is assessed as being unexpected, it becomes a suspected unexpected serious adverse reaction (SUSAR). Investigators should always check the current version of the SPC.

Safety reporting

The OUCRU CTU is responsible for the reporting of SUSARs and other SARs to the regulatory authorities and the research ethics committees. The following events will be reported to the relevant authorities in Vietnam and Indonesia: All unexpected SAEs, all SAEs judged to be related or possibly related to the trial intervention, and all deaths. All SAEs will be reported to OxtREC (Oxford Tropical Research Ethics Committee) in the annual review form and to the DMC in accordance to the DMC charter. An independent DMC will oversee the safety of the trial.

Interim analyses

Interim analyses are planned after 6 and 12 months of recruitment and yearly thereafter until the completion of the trial but the DMC has the authority to modify the frequency of interim analyses. At these interim analyses, the DMC will receive a report including unblinded summaries of baseline characteristics, the primary endpoint, and adverse events by treatment arm. The DMC will also review data from those enrolled into the DILI strategy ancillary study, in particular the incidence of acute hepatic failure in each of the management strategy arms.

Statistical analysis

Sample size justification – ACT HIV (main trial)

A previous trial of dexamethasone included 545 subjects with TBM and reported a reduced risk of death by 9 months associated with dexamethasone (hazard ratio of death 0.69 (95% CI 0.52–0.92)) and no evidence of heterogeneity with respect to HIV status though the number of HIV-infected patients was low (98 subjects) and none of them received antiretroviral therapy⁴. A recent TBM trial of intensified TBM treatment reported 9-month mortality in 349 HIV-infected patients of 40% in both

study arms and all subjects received dexamethasone⁷. Few additional deaths are expected to occur between months 9 and 12¹⁵. Assuming a target hazard ratio of 0.69 (corresponding to a mortality reduction from 52% to 40% in favour of dexamethasone), a total of 229 deaths need to be observed during the 12-month follow-up duration to obtain a power of 80% at the two-sided 5% significance level according to Schoenfeld's formula. To achieve this and allowing for a 5% loss-to-follow-up, a total of 520 HIV-infected subjects with TBM need to be recruited into the main trial.

Sample size justification – DILI strategy study

A review of 36 subjects who interrupted rifampicin and isoniazid because of clinical hepatitis or jaundice events from our previous trial⁷ gave the following data: the median (IQR) onset date of the DILI was 50 (15–84) days from initiation of anti-tuberculosis treatment, the median (IQR) duration of the rifampicin and isoniazid interruption was 16 (12–24) days, and 12 subjects subsequently died (8 of them within 60 days). Of note, the duration of the treatment interruption was <30 days for 32 (89%) of the 36 subjects and the remaining 4 subjects never re-started rifampicin or isoniazid but continued to receive alternative anti-tuberculosis treatment for >100 days. For the DILI strategy study we hypothesise that strategies 1 and 2 will result in a relative reduction in the duration of the treatment interruption of 50% for subjects with interruptions <30 days, but that they do not affect longer interruptions (as the corresponding subject might have permanent intolerance to rifampicin and isoniazid) or mortality. Based on simulations of hypothetical trials using re-sampling from the data described above, the hypothesised treatment effect, and the Wilcoxon rank sum test for analysis, we determined that the power to detect such an effect size with a sample size of at least 50 subjects per arm is >85%. Of note, given this is an ancillary and essentially 'opportunistic' study, we have chosen a liberal (i.e. not multiplicity corrected) two-sided significance level of 5% for each of the two primary comparisons of strategies 1 and 2, respectively, versus strategy 3. A total of 170 participants will be recruited to the DILI sub-study. The DILI sub-study has been powered for the combined analysis of ACT HIV (we anticipate 100 ACT HIV patients will consent to the DILI sub-study) and LAST ACT (a linked RCT of dexamethasone in HIV-uninfected adults with TBM stratified by LTA4H genotype, from which we anticipate 70 patients will enter the DILI sub-study)²².

Analysis populations – ACT HIV (main trial)

Patients in the main trial will be analysed according to their randomised arm as an intention-to-treat (ITT) analysis. In addition, the primary endpoint will be analysed in the per-protocol population, which will exclude the following patients: patients with a final diagnosis other than TBM, major protocol violations and those receiving less than 1 week of administration of the randomised study drug for reasons other than death. Published diagnostic criteria²³ will be applied to all enrolled participants at the end of the study when all mycobacterial culture results are available (Supplementary File 4). The criteria will sub-divide all cases into definite, probable and possible TBM, and those with an alternative diagnosis.

For the primary analyses of the main trial the second randomisation in the DILI strategy study will be ignored and the estimated dexamethasone treatment effect can thus be interpreted as an average effect across these three management strategies. We believe that this is justified because only approximately 100 (19%) subject are expected to be enrolled in the nested trial with roughly similar numbers from both arms, because the efficacy of the different management strategies is unlikely to depend on whether the patient received dexamethasone or not as it tests a very different intervention, and because the anticipated effect of the management strategy on survival is relatively small. However, in a supplementary analysis, we will also compare the primary endpoint between the treatment policies "dexamethasone treatment plus standard of care management of drug-related liver injury" vs. "placebo treatment plus standard of care management of drug-related liver injury" using an inverse probability weighting based analytical framework²⁴.

Analysis populations – DILI strategy study

All patients in the DILI strategy study will be analysed according to their randomised arm as an ITT analysis. The two primary comparisons are the comparisons of strategies 1 and 2, respectively, versus strategy 3 (with tests conducted at the unadjusted two-sided 5% significance level) and comparisons between strategies 1 and 2 will be exploratory only.

Primary endpoint analysis – ACT HIV (main trial)

The primary endpoint of the main trial is overall survival, i.e. time from randomisation to death, during 12 months of follow-up. Overall survival will be analysed with a Cox proportional hazards regression model with treatment as the only co-variate and stratification by TBM MRC severity grade at enrolment (I, II, or III) and country (Vietnam or Indonesia). The primary effect measure is the resulting hazard ratio comparing dexamethasone vs. placebo with a corresponding two-sided 95% confidence interval and p-value. The significance level of the associated two-sided test will be set to 5%. Kaplan-Meier plots and explicit survival estimates at 3, 6, 9, and 12 months of follow-up will also be calculated for the full populations and in the subgroups defined by TBM disease severity and country separately. The proportional hazards assumption will be formally tested based on scaled Schoenfeld residuals and visually assessed by a plot of the scaled Schoenfeld residuals versus transformed time. In case of a significant test, a formal comparison of the absolute risk of death at 12 months between the two groups will also be performed (using a Wald-type test based on Kaplan-Meier estimates at 12 months and associated standard errors using Greenwood's formula).

The homogeneity of the treatment effect on overall survival across subgroups will be assessed by subgroup analyses and formal tests of interaction between treatment and the following grouping variables: TBM MRC severity grade at enrolment (I, II, or III), country (Vietnam or Indonesia), drug resistance pattern (MDR-TB or rifampicin mono-resistance, isoniazid resistant non-MDR, no or other resistance), ART status at enrolment (ART naive, <3 months of ART, >3 months of ART), and CD4 cell count at enrolment. To obtain an adjusted treatment effect

estimate and to assess the effect of other covariates on survival, the primary endpoint will also be modelled using a multivariable Cox proportional hazards regression model including the following covariates (in addition to the treatment group): TBM MRC severity grade at enrolment, country, drug resistance pattern, ART status and CD4 cell count at enrolment. Multiple imputation will be used to handle missing covariates.

Primary endpoint analysis – DILI strategy study

For the analysis of the primary endpoint of the DILI strategy study, the non-parametric Wilcoxon rank sum test will be used for pairwise comparisons. An additional adjusted analysis (with adjustment for the initial randomisation, HIV-status, and the time from initial randomisation to the second randomisation) will be also be performed treating the outcome as an ordinal outcome and using a proportional odds logistic regression model (which can be interpreted as an extension of the Wilcoxon rank sum test).

Secondary endpoint analysis

For ACT HIV, neurological disability (assessed by modified Rankin scale) at 12 months will be compared between the two arms with a proportional odds logistic regression model with the treatment assignment as the main covariate and adjustment for TBM MRC severity grade, and country. The result will be summarised as a cumulative odds ratio with corresponding 95% CI and p-value. Patients with a missing 12-month disability assessment will be excluded from the main analysis but an alternative analysis based on multiple imputation (including disability assessments at earlier time points in the imputation model) will also be performed. Secondary time-to-event endpoints (time to neurological event or death, time to new AIDS event or death) will be analysed in the same way as the primary endpoint. The number of IRIS and HIV-associated malignancy events in each group will be summarised and the event rate calculated in each arm. Comparisons of the rates between the treatment arms will be based on a cause-specific proportional hazards model of the time to the first IRIS event (or HIV-associated malignancy, respectively) or death with treatment as the only covariate.

Analysis of adverse events

The number of patients with any adverse events and specific events, respectively, will be summarised and informally compared between the two treatment arms based on Fisher's exact test. The total number of adverse event episodes per patient will also be summarised and informally compared based on a quasi-Poisson regression model with treatment as the only covariate. The following subgroups of adverse events will also be separately summarised: grade 3&4 adverse events; serious adverse events; serious adverse events possibly, probably, or definitely related to the study drug; adverse events leading to TB treatment or ART interruptions. Grade 3&4 laboratory abnormalities will be summarised in the same way as clinical adverse events.

Adverse events due to DILI will be recorded for the DILI study but also for the primary intervention (dexamethasone or placebo). An increased number of adverse events in DILI arms 1 and 2 are not expected, but if they do occur these adverse events will be associated with both the DILI randomisation strategy and for the primary intervention arm in which they occurred.

Analysis of ancillary studies

Ancillary studies will be analysed within the same groups as allocated based on the dexamethasone intervention. Allocation to the intervention (dexamethasone or placebo) is important for each sub study, given the effect of corticosteroids on inflammatory pathways, diabetes control, Strongyloides infection, raised intracranial pressure and adrenal suppression. DILI study patients will be analysed in their DILI arm, but an additional adjusted analysis (with adjustment for the initial randomisation) will also be performed.

Baseline descriptive analyses

Baseline characteristics will be summarised as median (lower and upper quartiles) for continuous data and frequency (percentage) for categorical data. The amount of missing data for each baseline characteristic will also be displayed.

Ethical considerations

Treatment

All participants will receive the best available treatment of tuberculosis and HIV, following local and national guidelines. Corticosteroids are commonly prescribed drugs and there is widespread experience and expertise concerning their safe use. The choice (dexamethasone), dose, route of administration and duration of study treatment follows the international guidance for the treatment of HIV-uninfected participants with TBM. Previous trials have demonstrated the safety of this or similar regimens. In particular, adjunctive corticosteroids were not associated with increased gastro-intestinal bleeding in a meta-analysis of all published TBM trials, although participants in this trial will be carefully monitored for this event. There is a possibility dexamethasone may increase the risk of HIV-associated malignancies, which will also be monitored closely in the trial.

Confidentiality

Participants' confidentiality will be maintained throughout the trial. Participants will be assigned a trial identification number and this will be used on case report forms (CRFs); participants will not be identified by their name. The investigator will keep securely a participant trial register showing identification numbers, surnames and date of birth. The unique trial number will identify all laboratory specimens, case record forms, and other records and no names will be used, in order to maintain confidentiality. Data submitted to OUCRU CTU and samples sent to central testing facilities will be identified only by the trial number and participant initials.

Consent

Written informed consent must be obtained in order to enter into the trial and be randomised. If a participant lacks capacity, written consent must be obtained from a person with responsibility (e.g. family member/relative), in their own language before enrolment by the site PI or an appropriately trained doctor. All potential participants (or their families) will be given a participant information sheet clearly listing the risks and benefits of the trial. All potential participants (or their families) will be able to discuss participation with their consulting doctor who will be able to address questions not covered or arising from the participant information sheet. Incapacitated adults with TBM will

be eligible to enter the trial. These adults have more severe disease and therefore may benefit most from adjunctive dexamethasone. We anticipate around 70% of participants with TBM will lack capacity at the start of treatment. An option will be given to patients to enrol in the main study, but not the DILI strategy study.

If consent is provided by a relative, the participant should be consulted and consent recorded if and when they have the capacity to do so. If they are happy to remain in the trial, the participant should complete a participant consent form at this time. If they wish to withdraw from the trial, no further trial-related procedures will be performed, but data to this point would be used in analysis. Data from any participant who dies before regaining capacity (but whose family member has provided consent) will be included in analysis.

Protocol violations

All deviations from protocol will be addressed in source documents and reported to the OUCRU CTU.

Withdrawing from the trial

A participant (or their relative) is free to refuse to participate in or withdraw from all or any aspect of the trial, at any time and for any reason. If a participant chooses to discontinue their trial treatment they should always be followed up (providing they are willing) and they should be encouraged not to leave the whole trial. If they do not wish to remain on trial follow-up however, their decision must be respected and the participant will be withdrawn from the trial. Participants may change their minds about stopping trial follow-up at any time and re-consent to participation in the trial.

Data collection and storage

Clinical data and clinical laboratory data will be entered into CliRes, a 21 CFR Part 11-compliant data capture system provided by the OUCRU IT department. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Trial data will be recorded onto paper CRFs and entered into CliRes. The participants will be identified by a unique trial specific number and/or code in any database. The name and any other identifying detail will not be included in any trial data electronic file. CRFs, clinical notes and administrative documentation will be kept in a secure location and held for 15 years after the end of the trial. Clinical information will not be released without written permission, except as necessary for monitoring, auditing and inspection purposes. Electronic data will be kept for at least 20 years at the OUCRU CTU.

SPIRIT checklist

A SPIRIT checklist for this trial protocol is attached ([Supplementary File 5](#)).

Trial Committees

A Trial Management Group (TMG) will be formed to conduct the day-to-day management of the trial at the OUCRU CTU. This will include the CI, Head of OUCRU CTU, Trial

Statistician, Clinical Project Manager, Trial Manager, Data Manager and Jakarta trial coordinator. The group will meet at least once per month, although may meet more or less often as required. The TSC has membership from the TMG plus independent members (Professor Nicholas Paton (Infectious Diseases Physician and Clinical Trialist, National University of Singapore, Singapore), Professor Ben Marais (Senior Tuberculosis Researcher and Trialist, University of Sydney, Australia), Dr Truong Huu Khanh (Infectious Diseases Physician, Paediatric Hospital Number 1, Ho Chi Minh City, Vietnam)), including the Chair (Professor Robert Wilkinson (Honorary Professor and Director Wellcome Centre for Infectious Diseases Research in Africa, University of Cape Town, South Africa)). The role of the TSC is to provide overall supervision for the trial and provide advice through its independent chair. The ultimate decision for the continuation of the trial lies with the TSC. The DMC (Professor Sarah Walker (DMC Chair, Senior Statistician and Clinical Trialist, MRC Clinical Trials Unit, University College London), Professor Graeme Meintjes (Senior Infectious Diseases/HIV Physician, University of Cape Town, South Africa) and Professor Nina Ruslami (Senior TBM Clinician and Researcher, Universitas Padjadjaran, Bandung, Indonesia)), will advise the TSC and can recommend premature closure or reporting of the trial, or that recruitment be discontinued or modified. The DMC is independent from the sponsor. Access to interim data and results will be confidential and strictly limited to the DMC and results (except for the recommendation) will not be communicated to the outside and/or clinical investigators involved in the trial. This trial is sponsored by The University of Oxford (Contact: University of Oxford, Research Services, University Offices, Wellington Square, Oxford OX1 2JD, Tel +44 (0) 1865 282585).

Data dissemination

Manuscripts arising from the trial will, wherever possible, be submitted to peer-reviewed journals which enable Open Access via UK PubMed Central (PMC) within six months of the official date of final publication. In line with research transparency and greater access to data from trials OUCRU's clinical trials are registered at ClinicalTrials.gov and a data sharing policy is in place. Data exchange complies with Information Governance and Data Security Policies in all of the relevant countries.

Discussion

TBM remains the most severe form of tuberculosis, and is especially common in those infected with HIV. Whether corticosteroids confer a survival benefit in HIV-infected patients remains uncertain, with the current evidence base for using corticosteroids in this context restricted to 98 adults recruited to a trial in Vietnam^{6,13}. The ACT HIV trial aims to determine whether dexamethasone is a safe and effective addition to the first 6–8 weeks of anti-tuberculosis treatment of TBM. ACT HIV will be performed in parallel with a randomised double blind placebo controlled trial of adjunctive dexamethasone in HIV-uninfected Vietnamese adults stratified by Leukotriene A4 hydrolase (LTA4H) genotype (LAST ACT, clinical trial registration NCT03100786)²². Both trials will recruit to seven ancillary studies, including a

study to investigate alternative management strategies in a subset of patients who develop DILI, enabling safe continuation of rifampicin and isoniazid therapy whenever possible. In recruiting the target 520 patients into the main trial, this trial has the opportunity to also study the following: Host and bacterial genetic determinants of treatment response; Impact of dexamethasone on CSF inflammation and gross cerebral pathology; Influence of diabetes mellitus on presentation and response to treatment; Influence of *Strongyloides* infection on presentation and response to treatment. Pathophysiology and treatment of hyponatraemia and raised intracranial pressure; Dexamethasone induced adrenal suppression. These data will be valuable in guiding the management of adjunctive corticosteroid therapy in HIV-infected individuals.

Trial status

Trial protocol version 1.4, 1st August 2017. Estimated recruitment start date 1st June 2017. Estimated time for recruitment is 4 years.

Ethical statement

The trial has ethics approval from the Oxford Tropical Research Ethics Committee (approval number 36-16), the Ethics Committees of the Hospital for Tropical Diseases (approval number CS/ND/16/37) and Pham Ngoc Thach Hospital (approval number CS/PT/16/07), the Vietnam Ministry of Health, and Faculty of Medicine University of Indonesia (17-01-0066). Protocol version 1.4 has ethics approval from the Oxford Tropical Research Ethics Committee, the Ethics Committee of the Hospital for Tropical Diseases, the Ethics Committee of Pham Ngoc Thach Hospital, the Vietnam Ministry of Health, and the Faculty of Medicine University of Indonesia.

Informed consent will be obtained for all study participants.

Data availability

No data are associated with this article.

Disclosures

Due to the linked nature of this trial, some sections of this protocol also form part of the linked RCT LAST ACT (Trial registration number: [NCT03100786](#)), which has also been submitted to *Wellcome Open Research*²². LAST ACT is a parallel group, randomised (1:1), double blind, placebo-controlled, multi-centre Phase III non-inferiority trial, comparing dexamethasone versus placebo for 6–8 weeks in addition to standard anti-tuberculosis treatment in HIV-uninfected patients with TBM stratified by LTA4H genotype. The 7 ancillary studies in ACT HIV are also recruited to through the LAST ACT trial. As such the hypotheses, design, methods, sample size justification; analysis plans and endpoints of these ancillary studies will also be described in the LAST ACT trial, and will appear identically here. ACT HIV follows the same OUCRU protocols and local / national guidelines as LAST ACT, therefore randomisation, blinding and unblinding procedures, adverse event and safety reporting, ethics and confidentiality sections also appear identically here for ACT HIV as for the LAST ACT trial.

Competing interests

No competing interests were disclosed.

Grant information

The trial is supported by the Wellcome Trust [110179], an Investigator Award to Professor Guy Thwaites.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgements

We would like to acknowledge the important contributions of all doctors nurses and patients who will make the trial a success. We would like to thank OUCRU CTU and EOCRU administrative staff for their help in conducting the study.

Supplementary material

Supplementary File 1. Modified MRC grade of TBM.

[Click here to access the data.](#)

Supplementary File 2. IRIS diagnostic criteria.

[Click here to access the data.](#)

Supplementary File 3. Current standard of care in DILI.

[Click here to access the data.](#)

Supplementary File 4. TBM diagnostic criteria.

[Click here to access the data.](#)

Supplementary File 5. SPIRIT checklist.

[Click here to access the data.](#)

Supplementary File 6. ACT HIV consent form for HTD Vietnam.

[Click here to access the data.](#)

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Open Peer Review


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Version 2

Referee Report 17 September 2018

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A study on adjunctive dexamethasone for the treatment of HIV-infected adults with tuberculous meningitis (ACT HIV) is much needed and could prove to be of immense benefit.

- *The exclusion criteria includes:*

'b) More than 6 consecutive days of two or more drugs active against immediately before screening.

c) More than 3 consecutive days of any type of orally or intravenously administered corticosteroid immediately before randomisation"

1. It would help to mention an interval of how many hours-days is required to be eligible for the study in case the person has received any of the afore-mentioned drugs prior to the study.

2. Does the inclusion criteria include persons with TBM as a part of disseminated TB?

- *The Ancillary study 6: Pathophysiology and treatment of hyponatraemia and raised intracranial pressure*
- does not specify what modalities will be used to determine the best method of treatment.

4. Will the ART be modified if the patient develops DILI?

5. Interactions with Rifampicin and EFV are also seen. Are you going to monitor the TDM of efavirenz when using rifampicin in these patients?

6. What about co-infection with HBV and HCV? Are you going to include these patients? It is not mentioned in the inclusion or exclusion criteria.

7. Other co-infections in HIV infected (which are non-neurological) is also not mentioned. Some patients do have systemic fungal infections such as esophageal candidiasis and histoplasmosis. Giving dexamethasone in these patients will be a problem. Are you going to include these patients as well as?

8. Survival in HIV infected patients with TBM will also depend on baseline CD4 count and HIV viral load. So that may be a confounding factor when analysing the data. This issue needs to be addressed.

9. In case of DILI in the intensive phase, a regime of Lfx, E and aminoglycoside may not be enough. Would you consider addition of Linezolid or any other drug that is not hepatotoxic and does not have interaction with the ART.

Overall, the study protocol is well written.

Is the rationale for, and objectives of, the study clearly described?

Yes

Is the study design appropriate for the research question?

Yes

Are sufficient details of the methods provided to allow replication by others?

Yes

Are the datasets clearly presented in a useable and accessible format?

Yes

Competing Interests: No competing interests were disclosed.

We have read this submission. We believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Referee Report 17 July 2018

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We thank authors for their reply to our concerns

However, we still have some concerns regarding the protocol;

1. Q 1. We still have significant concerns about the DILI substudy arm1 for safety issues.

2. Q.4. We disagree with the authors that imaging is not being done as a standard for all patients. As a part of the study, at least a CT scan and MRI where necessary among all patients should be mandatory in view that all of them are HIV positive patients and could have infection other than TB as well as to detect complications.

3. Q7. Since the inclusion criteria is patients with HIV, we believe that most patients would hopefully be on treatment already at the time of their presentation with suspected TBM. We understand that ART is typically advised after an interval of starting ATT among patients with and TBM newly diagnosed HIV or known HIV without any ART therapy, in view of the previous trial results as quoted by the author. However steroid was not the intervention in that trial. Our concern whether an earlier ART is to be

considered was because one group of patients are being given dexamethasone, which may have a concern of worsening the underlying HIV disease. The best evidence from the previous trial and recommendations of experts should be taken to decide about the best time to start ART in this trial, as we understand that this a RCT and one group is free from dexamethasone which of course may complicate the study design. However, further expert opinion is warranted.

Competing Interests: No competing interests were disclosed.

We have read this submission. We believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Author Response 25 Jul 2018

Joseph Donovan, Oxford University Clinical Research Unit, Ho Chi Min City, Vietnam

Question 1

We feel we have already addressed this question and no further details are given as to why there are safety concerns regarding arm 1 of the DILI sub-study. There is no evidence to suggest that DILI arm 1 is unsafe, and the purpose of clinical research such as this is to produce high quality evidence to support our clinical treatment. We do not know that it is safer to stop essential first line anti-tuberculous drugs at an ALT threshold of $>5\times$ ULN, and change to less effective anti-tuberculous therapy. This is frequently done but we do not know it is the safer approach in TBM. As previously explained, it is anticipated that arm 1 (continue all 4 first line anti-TB drugs) and arm 2 (stop only pyrazinamide) of the DILI study will result in less adverse events due to the continuation of the best and most appropriate anti-TB drugs. There are clear safety thresholds where a patient in DILI arms 1 or 2 moves to arm 3. Patients are reviewed daily and LFTs, including INR, are checked regularly. This trial has received ethical approval from the Oxford Tropical Research Ethics Committee, the Ethics Committees of the Hospital for Tropical Diseases and Pham Ngoc Thach Hospital, the Vietnam Ministry of Health, and Faculty of Medicine University of Indonesia. An independent data monitoring committee will review unblinded trial data at interim analysis points and advise on any safety issues. We believe the DILI sub-study is important, and there is no evidence base to say that arm 1 is unsafe.

Question 2

CT or MRI brain imaging can be important in the management of a patient with TBM, but it is not always available. It may be ideal to have imaging for every patient we treat but this is not always possible in all settings. Clinical trials cannot only be performed in sites where gold standard level testing is available for every patient. Where availability of imaging is limited, imaging must be done on a case-by-case basis where it is required.

Question 3

The experience of the reviewers suggests that most patients would be on anti-retroviral treatment at the time of presentation with TBM, however this is not the case in our setting. We do not believe that corticosteroid therapy will worsen HIV infection, and corticosteroids are often given to HIV co-infected individuals for a multitude of reasons. Furthermore, this is a double blind trial and physicians will not know who is treated with dexamethasone. The reviewers say that "the best evidence from the previous trial and recommendations of experts should be taken to decide about the best time to start ART in this trial". Our protocol states that ART in both Ho Chi Minh City and Jakarta is often commenced 6–8 weeks after the start of anti-tuberculosis treatment, "with the

precise timing left to the discretion of the attending physician". Our trial physicians also follow local expert guidelines. We do not believe that in the absence of additional clinical trial data further expert guidance is needed.

Yours Sincerely

Professor Guy Thwaites (Senior author and study Chief Investigator)

Dr Joseph Donovan (First author and Research Fellow)

Competing Interests: No competing interests were disclosed.

Version 1

Referee Report 08 June 2018

doi:10.21956/wellcomeopenres.15222.r33086



Rohit Bhatia¹, Manish Modi²

¹ Department of Neurology, All India Institute of Medical Sciences (AIIMS), New Delhi, Delhi, India

² Postgraduate Institution of Medical Education and Research, Chandigarh, India

The protocol is well written. Our concerns and queries are outlined below.

1. DILI study: A concern to the reviewers is the co-study of DILI related interventions within the same trial. Since these patients are HIV infected, with adjuvant therapies and co-morbidities, further randomization to the groups may increase the likelihood of adverse events and outcomes especially with the allocation strategies planned.
2. Is the DILI study strategy 1 safe. Could the authors consider strategy 2 and 3 only.
3. Many ancillary studies are planned from the same data. Will the patients be analyzed within the same groups as allocated based on the dexamethasone intervention allocation. The same is not clear.
4. The authors have planned to do imaging at intervals. The authors may kindly clarify whether imaging is being done at baseline for all patients for exclusion of diagnosis other than TBM and also to ascertain presence of features suggestive of TBM and features like hydrocephalus, tuberculomas, infarcts etc., as this is extremely important. An imaging at the end of the study may be desirable to ascertain resolution of the disease.
5. The authors have planned on an ATT regimen excluding streptomycin. What about patients with basal visual complications. Is there a provision to alter the regimen by replacing streptomycin instead of ethambutol in such group of patients?
6. The authors plan to use RHE regimen in the maintenance phase. Would authors consider to use pyrazinamide instead of ethambutol in the maintenance phase, considering the higher CNS bioavailability of this drug.
7. The authors plan to start ART after 4-6 weeks of the diagnosis. Considering that steroids are being used in this trial, would the authors consider an earlier ART intervention than planned? Is there a shorter period that is safe? What about a patient who is already a diagnosed case of HIV on ART. Will he/she be excluded from the study.

8. Is Pregnancy an exclusion criteria.
9. It is reasonable to exclude presence of non-tuberculous mycobacteria and also presence of MDR TB at baseline by best available modalities to ascertain diagnostic certainty and therapeutic responsiveness.
10. Will participants with geneXpert suggestive of rifampicin resistance be modified in the ATT regimen or will be excluded from the study?
11. Other complications like opto-chiasmatic arachnoiditis and myelitis should also be included in the outcomes as dexamethasone use is reported to help in these complications.

Is the rationale for, and objectives of, the study clearly described?

Yes

Is the study design appropriate for the research question?

Yes

Are sufficient details of the methods provided to allow replication by others?

Partly

Are the datasets clearly presented in a useable and accessible format?

Yes

Competing Interests: No competing interests were disclosed.

We have read this submission. We believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Author Response 15 Jun 2018

Joseph Donovan, Oxford University Clinical Research Unit, Ho Chi Min City, Vietnam

Dear Dr Bhatia and Dr Modi,

Thank you very much reviewing our ACT HIV trial protocol. I would like to address each of your points individually.

Question 1

DILI study: A concern to the reviewers is the co-study of DILI related interventions within the same trial. Since these patients are HIV infected, with adjuvant therapies and co-morbidities, further randomization to the groups may increase the likelihood of adverse events and outcomes especially with the allocation strategies planned.

Author reply

DILI related interventions are conducted within the same trial, but the DILI strategies (specifically arms 1 and 2, compared with the routine care arm 3) are not expected to increase adverse events. It is in fact anticipated that arm 1 (continue all 4 first line anti-TB drugs) and arm 2 (stop only pyrazinamide) of the DILI study will result in less adverse events due to the continuation of the best and most appropriate anti-TB drugs. With regard to liver function, this will be closely monitored. We accept that patients in the DILI study may be on concurrent ART. However, DILI occurs more

frequently in individuals with HIV therefore it is very important that DILI interventions are assessed in an HIV positive group, to avoid unnecessary cessation of first line anti-TB drugs if arms 1 and / or 2 are effective. From a safety perspective, if a patient develops DILI, prior randomisation to dexamethasone or placebo should not affect DILI in an adverse way.

If DILI develops ART should not be stopped until all other management has been tried (including interrupting anti-tuberculosis drugs according to the randomised schedule). HIV specialists and liver specialists should be consulted at this point. Patients will receive regular clinical and blood test monitoring, and an independent data monitoring committee will review unblinded trial data at interim analysis points. Adverse events due to DILI will be recorded for the DILI study but also for the primary intervention (dexamethasone or placebo). An increased number of adverse events in DILI arms 1 and 2 are not expected, but if they do occur it will be made clear in the analysis if they were associated with DILI randomisation, although these adverse events will also be reported for the primary intervention arm in which they occurred.

Question 2

Is the DILI study strategy 1 safe? Could the authors consider strategy 2 and 3 only?

Author reply

The authors believe that DILI strategy 1 is safe, and this sub study has received ethical approval. Patients will undergo regular clinical and blood test monitoring, and there is a clear procedure to follow for moving patients from strategy 1 to strategy 3. An independent data monitoring committee will review unblinded data from this trial at interim analysis time points and advise the trial steering committee on any safety issues. The authors are not aware of any evidence suggesting a lack of safety of arm 1 of the DILI study compared with arm 3. Stopping rifampicin isoniazid and pyrazinamide (strategy 3) may in fact be the least safe option in TBM due to discontinuation of the most important drugs for the treatment of brain infection.

Question 3

Many ancillary studies are planned from the same data. Will the patients be analyzed within the same groups as allocated based on the dexamethasone intervention allocation. The same is not clear.

Author reply

Ancillary studies will be analysed within the same groups as allocated based on the dexamethasone intervention allocation. Allocation to the intervention (dexamethasone or placebo) is important for each sub study, given the effect of corticosteroids on inflammatory pathways, diabetes control, Strongyloides infection, raised intracranial pressure and adrenal suppression. For the DILI study patients will be analysed in their DILI arm, but an additional adjusted analysis (with adjustment for the initial randomisation) will also be performed.

Question 4

The authors have planned to do imaging at intervals. The authors may kindly clarify whether imaging is being done at baseline for all patients for exclusion of diagnosis other than TBM and also to ascertain presence of features suggestive of TBM and features like hydrocephalus, tuberculomas, infarcts etc., as this is extremely important. An imaging at the end of the study may be desirable to ascertain resolution of the disease.

Author reply

All patients recruited to this study at HTD in Vietnam will have MRI brain imaging at day 0, day 60

and at 1 year (assuming they consent to the MRI ancillary study). At the other sites, brain imaging is performed based upon the routine care offered for brain infection at that site. In many cases brain imaging will be performed in order to establish the diagnosis and investigate for complications. However it is accepted that brain imaging is not always readily available at all study sites, and this study does not mandate brain imaging for every patient. In the context of an unclear clinical presentation, or clinical signs where brain imaging is required, the study team will strongly support the decision of the treating physician to conduct brain imaging.

Question 5

The authors have planned on an ATT regimen excluding streptomycin. What about patients with basal visual complications. Is there a provision to alter the regimen by replacing streptomycin instead of ethambutol in such group of patients?

Author reply

First line anti-tuberculosis treatment will follow current Vietnamese and Indonesian national guidelines, where rifampicin, isoniazid, pyrazinamide and ethambutol will be given for at least the first 2 months of treatment where there is no drug resistance. Patients with visual complications will discontinue ethambutol and an alternative drug will be used in its place. The decision of this 4th drug will be made by the treating physician, and is not mandated by the trial. In practice it will be either a fluoroquinolone or streptomycin.

Question 6

The authors plan to use RHE regimen in the maintenance phase. Would authors consider to use pyrazinamide instead of ethambutol in the maintenance phase, considering the higher CNS bioavailability of this drug?

Author reply

First line anti-tuberculosis treatment will follow current Vietnamese and Indonesian national guidelines. Rifampicin, isoniazid, pyrazinamide and ethambutol will be given for at least the first 2 months of treatment where there is no drug resistance. Pyrazinamide will then be stopped and rifampicin, isoniazid and ethambutol will be given until month 12. This trial does not seek to assess alternative anti-tuberculous drug treatment strategies and anti-tuberculous therapy will follow national guidelines.

Question 7

The authors plan to start ART after 4-6 weeks of the diagnosis. Considering that steroids are being used in this trial, would the authors consider an earlier ART intervention than planned? Is there a shorter period that is safe? What about a patient who is already a diagnosed case of HIV on ART. Will he/she be excluded from the study?

Author reply

Immediate commencement of ART (compared with ART commenced after 2 months) in TBM has been associated with increased grade 4 adverse events, supporting deferred commencement of ART (Torok et al. Clin Infect Dis. 2011 Jun; 52(11): 1374–1383). The optimal time to start ART in TBM is not known, depends upon TBM disease severity and CD4 count, and varies between centres regarding routine practice. Exactly when to start ART is decided upon by the treating physician, although this is often between 6 and 8 weeks after TBM diagnosis. There is no evidence to guide whether a shorter period (for example 2 weeks) is safe in TBM. Only 50% of patients in this trial receive corticosteroids, and as the trial is double blinded these patients cannot be

identified. Therefore adjunctive corticosteroid use cannot be used to support starting ART earlier. A patient with known HIV already on ART will continue this ART when they enter the trial, and during the trial.

Question 8

Is Pregnancy an exclusion criteria?

Author reply

No, pregnancy is not an exclusion criteria.

Question 9

It is reasonable to exclude presence of non-tuberculous mycobacteria and also presence of MDR TB at baseline by best available modalities to ascertain diagnostic certainty and therapeutic responsiveness.

Author reply

Exclusion of non-tuberculous mycobacteria infection and MDR TBM would be an alternative way to run this trial. However we would like to enroll those adult HIV-infected patients who have a clinical diagnosis of TBM and assess the effect of corticosteroids. MDR TBM patients are an important part of this group and will be included. If infection with non-tuberculous mycobacteria is suspected then the patient will not be enrolled (this would be an alternative brain infection, and this is an exclusion criteria). If non-tuberculous mycobacteria are later identified as the cause of disease (instead of TBM) the patient will still form part of the intention to treat analysis in the arm in which they were randomised.

Question 10

Will participants with geneXpert suggestive of rifampicin resistance be modified in the ATT regimen or will be excluded from the study?

Author reply

Patients with a positive GeneXpert result consistent with rifampicin resistance will receive MDR TBM treatment as per national guidelines, in conjunction with a regional specialist. They will not be excluded from the main study. They will however not be eligible for the DILI study.

Question 11

Other complications like opto-chiasmatic arachnoiditis and myelitis should also be included in the outcomes as dexamethasone use is reported to help in these complications.

Author reply

If these conditions are detected they will be recorded. However they do not form part of the outcomes of this trial (unless they lead to focal neurological deficits or a decrease in GCS as per the criteria in the protocol).

Competing Interests: No competing interests were disclosed.

Referee Report 26 March 2018

doi:10.21956/wellcomeopenres.15222.r32220

**Ravindra K Garg** 

Department of Neurology, King George's Medical University, Lucknow, Uttar Pradesh, India

Adjunctive dexamethasone for the treatment of HIV-infected adults with tuberculous meningitis is a pertinent issue which still need to be answered. The protocol is well written and the study will be conducted by known experts of the field. How will authors deal with complications of TBM, like hydrocephalus, stroke, tuberculomas and vision loss at inclusion and follow up, is not very clear cut. A neuroimaging at inclusion I suggest should be done in all cases, other wise many of these complications will be missed. Is there any provision for shunt surgery?

Is the rationale for, and objectives of, the study clearly described?

Yes

Is the study design appropriate for the research question?

Yes

Are sufficient details of the methods provided to allow replication by others?

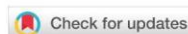
Yes

Are the datasets clearly presented in a useable and accessible format?

Yes

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.



STUDY PROTOCOL

Adjunctive dexamethasone for the treatment of HIV-uninfected adults with tuberculous meningitis stratified by Leukotriene A4 hydrolase genotype (LAST ACT): Study protocol for a randomised double blind placebo controlled non-inferiority trial [version 1; peer review: 2 approved]

Joseph Donovan ^{1,2}, Nguyen Hoan Phu ³, Le Thi Phuong Thao ¹,
 Nguyen Huu Lan ⁴, Nguyen Thi Hoang Mai ³, Nguyen Thi Mai Trang ⁴,
 Nguyen Thi Thu Hiep ¹, Tran Bao Nhu ³, Bui Thi Bich Hanh ³, Vu Thi Phuong Mai ¹,
 Nguyen Duc Bang ⁴, Do Chau Giang ⁴, Dang Thi Minh Ha ⁴, Jeremy Day ^{1,2},
 Nguyen TT Thuong ^{1,2}, Nguyen Nang Vien ⁴, Ronald B. Geskus ^{1,2},
 Tran Tinh Hien ¹, Evelyn Kestelyn ^{1,2}, Marcel Wolbers ¹,
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Abstract

Background: Tuberculosis kills more people than any other bacterial infection worldwide. In tuberculous meningitis (TBM), a common functional promoter variant (C/T transition) in the gene encoding leukotriene A4 hydrolase (LTA4H), predicts pre-treatment inflammatory phenotype and response to dexamethasone in HIV-uninfected individuals. The primary aim of this study is to determine whether LTA4H genotype determines benefit or harm from adjunctive dexamethasone in HIV-uninfected Vietnamese adults with TBM. The secondary aim is to investigate alternative management strategies in individuals who develop drug induced liver injury (DILI) that will enable the safe continuation of rifampicin and isoniazid therapy.

Methods: We will perform a parallel group, randomised (1:1), double blind, placebo-controlled, multi-centre Phase III non-inferiority trial, comparing dexamethasone versus placebo for 6-8 weeks in addition to standard anti-tuberculosis treatment in HIV-uninfected patients with TBM stratified by LTA4H genotype. The primary endpoint will be death or new neurological event. The trial will enrol approximately 720 HIV-uninfected adults with a clinical diagnosis of TBM, from two hospitals in Ho Chi Minh City, Vietnam.

Open Peer Review

Reviewer Status

	Invited Reviewers	
	1	2
version 1		
20 Mar 2018	report	report

- Ravindra Kumar Garg** , King George's Medical University, Lucknow, India
- Katalin A. Wilkinson** , University of Cape Town, Observatory, South Africa
The Francis Crick Institute, London, UK

Any reports and responses or comments on the article can be found at the end of the article.

640 participants with CC or CT- LTA4H genotype will be randomised to either dexamethasone or placebo, and the remaining TT- genotype participants will be treated with standard-of-care dexamethasone. We will also perform a randomised comparison of three management strategies for anti-tuberculosis DILI. An identical ancillary study will also be performed in the linked randomised controlled trial of dexamethasone in HIV-infected adults with TBM (ACT HIV).

Discussion: Previous data have shown that LTA4H genotype may be a critical determinant of inflammation and consequently of adjunctive anti-inflammatory treatment response in TBM. We will stratify dexamethasone therapy according to LTA4H genotype in HIV-uninfected adults, which may indicate a role for targeted anti-inflammatory therapy according to variation in LTA4H C/T transition. A comparison of DILI management strategies may allow the safe continuation of rifampicin and isoniazid.

Keywords

Tuberculous meningitis, HIV-uninfected, Dexamethasone, Drug induced liver injury, LTA4H, Adrenal suppression, Hyponatraemia



This article is included in the [Oxford University Clinical Research Unit \(OUCRU\) gateway](#).

Corresponding author: Joseph Donovan (jdonovan@oucru.org)

Author roles: Donovan J: Methodology, Supervision, Writing – Original Draft Preparation; Phu NH: Methodology, Supervision; Thao LTP: Formal Analysis; Lan NH: Methodology, Project Administration, Supervision; Mai NTH: Investigation, Methodology; Trang NTM: Methodology, Project Administration, Supervision; Hiep NTT: Investigation, Project Administration; Nhu TB: Investigation, Project Administration; Hanh BTB: Investigation, Project Administration; Mai VTP: Project Administration; Bang ND: Methodology, Project Administration, Supervision; Giang DC: Methodology, Project Administration, Supervision; Ha DTM: Methodology, Project Administration, Supervision; Day J: Methodology; Thuong NT: Methodology; Vien NN: Investigation, Supervision; Geskus RB: Formal Analysis; Hien TT: Methodology, Supervision; Kestelyn E: Methodology, Supervision, Writing – Review & Editing; Wolbers M: Formal Analysis; Chau NVV: Methodology, Supervision; Thwaites GE: Conceptualization, Funding Acquisition, Supervision, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

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Introduction

Background

Tuberculous meningitis (TBM) is the most severe form of tuberculosis, resulting in death or neurological disability in approximately 40% of HIV-uninfected sufferers, and 70% of those co-infected with HIV. Effective anti-tuberculosis chemotherapy has been available for more than 50 years, yet tuberculosis kills more people than any other single bacterial infection worldwide. Diagnosing TBM is difficult and depends upon detecting low numbers of *M. tuberculosis* bacteria in cerebrospinal fluid (CSF). Rifampicin (R), isoniazid (H), pyrazinamide (Z) and ethambutol (E) are the first-line antibiotics for all forms of drug-susceptible tuberculosis. These agents, used daily and in combination, kill the bacteria and prevent the emergence of resistance. There is concern that the blood-brain barrier reduces the concentrations of anti-tuberculosis drugs in the brain which may attenuate both bacterial killing and clinical response¹. The most recent Cochrane meta-analysis and systematic review of adjunctive corticosteroids for TBM² concluded that corticosteroids likely reduced death from TBM in HIV-uninfected individuals in the short term (up to 18 months of follow-up), but had no clear impact on longer term neurological disability or survival.

Complications of TBM

Hydrocephalus, stroke, and tuberculoma formation are important and common complications of TBM³, however little evidence exists to guide their optimal management. Drug induced hepatitis associated with anti-tuberculosis treatment occurs in around 10% of HIV-uninfected patients⁴, and can be attributed to rifampicin, isoniazid or pyrazinamide. Determining which drug is responsible is difficult. Corticosteroid therapy leads to hyperglycaemia, and during treatment of TBM dexamethasone may worsen, or reveal, diabetes. Severe hyponatraemia (low plasma sodium), high temperature and CO₂ retention all exacerbate raised intracranial pressure and require active management. The role of corticosteroids in adrenal suppression in TBM requires further investigation.

The role of LTA4H

How corticosteroids improve survival in HIV-uninfected patients with TBM, and whether they do so in all HIV-uninfected patients, remains uncertain and is the focus of the LAST ACT trial. A common functional promoter variant (C/T transition) in the gene encoding LTA4H, which determines the balance of pro- and anti-inflammatory eicosanoids, appears to predict pre-treatment inflammatory phenotype and response to dexamethasone in HIV-uninfected participants^{5,6}. In humans, LTA4H rs17525495 allele homozygosity (TT and CC respectively) was associated with susceptibility to mycobacterial infection, albeit with opposite inflammatory states - high for the TT-genotype and low for the CC-genotype⁵. Heterozygotes (CT) had an intermediate inflammatory response, and were more likely to survive TBM⁵. A retrospective study, analysing HIV-uninfected Vietnamese adults with TBM enrolled into an earlier randomised controlled trial of adjunctive dexamethasone⁷ and prospective TBM observational studies, found that the survival benefit of dexamethasone was restricted to the hyper-inflammatory LTA4H TT-genotype patients, with possible harm suggested in hypo-inflammatory CC-genotype patients⁸. This suggests LTA4H genotype may

be a critical determinant of inflammation and consequently of adjunctive anti-inflammatory treatment response.

Hypotheses

Dexamethasone has been shown to improve survival in HIV-uninfected individuals with TBM². Previous data strongly suggests hyperinflammatory LTA4H TT-genotype patients with TBM benefit from dexamethasone, and that adjunctive dexamethasone may not benefit, and may cause harm, when given to patients with LTA4H CT or CC-genotype. **Our primary hypothesis is that dexamethasone has no benefit in patients with CC or CT genotype for adults with TBM.** We regard the benefit of dexamethasone in patients with LTA4H TT-genotype as established and will hence not randomise them. In contrast, patients with CT or CC-genotypes will be randomised in a practice-defining randomised controlled trial (RCT) to assess whether placebo is non-inferior or even superior to dexamethasone in these patients. Our secondary hypothesis is that current guidelines for the management of anti-tuberculosis DILI in those with TBM result in the premature cessation of rifampicin and isoniazid (the critical active drugs in early therapy) and place patients at unnecessary risk of TBM-related death and disability.

Study aims

Primary aim

Our primary aim is to determine whether LTA4H genotype, defined at randomisation, determines dexamethasone's clinical effectiveness when added to the first 6-8 weeks of anti-tuberculosis treatment of TBM with dexamethasone duration depending on Medical Research Council (MRC) grade ([Supplementary File 1](#)) at the start of treatment. In making this assessment we not only determine whether in LTA4H CT or CC-genotype patients placebo is non-inferior to dexamethasone with respect to survival and the incidence of new neurological events, but also with respect to disability assessed by the modified Rankin scale ([Table 1](#)), the frequency of severe and serious adverse events, and the need for rescue corticosteroids.

Secondary aims

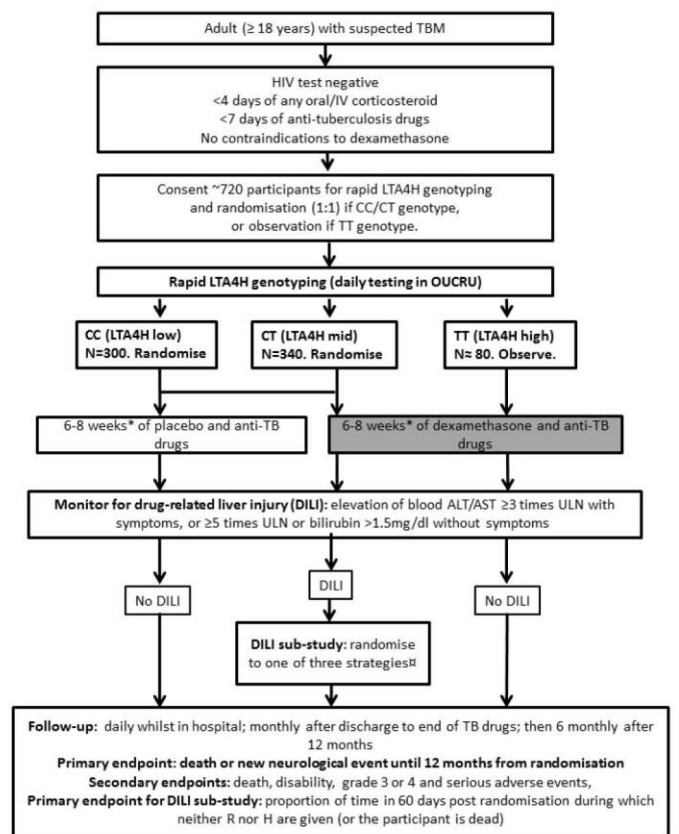
Our secondary aim is to investigate alternative management strategies in a subset of patients who develop DILI that will enable the safe continuation of rifampicin and isoniazid therapy whenever possible.

Design and setting

LAST ACT is a parallel group, randomised (1:1), double blind, placebo-controlled, multi-centre Phase III non-inferiority trial, comparing dexamethasone versus placebo for 6-8 weeks in addition to standard anti-tuberculosis treatment in HIV-uninfected patients with TBM stratified by LTA4H genotype. The trial will be set in two hospitals in Ho Chi Minh City, Vietnam. The trial will enrol approximately 720 HIV-uninfected adults (≥18 years) with a clinical diagnosis of TBM, as judged by the attending physician, requiring immediate anti-tuberculosis treatment. Amongst these, 640 participants with CT or CC-LTA4H genotype will be randomised to either dexamethasone or placebo. The remaining participants (approximately 80) with TT-genotype will be treated with standard-of-care dexamethasone. The trial schema is shown in [Figure 1](#).

Table 1. Modified Rankin scale.

Score	Description
0	No symptoms
1	Minor symptoms not interfering with lifestyle
2	Symptoms that lead to some restriction in lifestyle, but do not interfere with the patients ability to look after themselves
3	Symptoms that restrict lifestyle and prevent totally independent living
4	Symptoms that clearly prevent independent living, although the patient does not need constant care and attention
5	Totally dependent, requiring constant help day and night
6	Death



Must be confirmed; withdraw if confirmatory tests are negative

† Relatives can provide consent if the patient is incapacitated

* MRC Grade I: 6 weeks; MRC Grade II and III: 8 weeks

‡ 1. Raising threshold for stopping drugs; 2. stopping Z alone; 3. stopping RHZ with gradual reintroduction

Figure 1. Trial Schema for LAST ACT trial (main trial).

An ancillary DILI strategy study will perform a randomised comparison of management strategies in DILI early in anti-tuberculosis treatment (the intensive phase). We will perform an open, randomised comparison of three management strategies with the aim of demonstrating which strategy results in the least interruption in rifampicin and isoniazid treatment. All patients enrolled in the main trial will be eligible to take part in this

study, with the exception of those known to have TBM caused by isoniazid resistant or MDR *M. tuberculosis*. An identical ancillary study will also be performed in the linked RCT of dexamethasone in HIV-infected adults with TBM (ACT HIV)⁸. The results from both trials will be reported together, after the primary results from both trials have been published. This trial schema is shown in Figure 2.

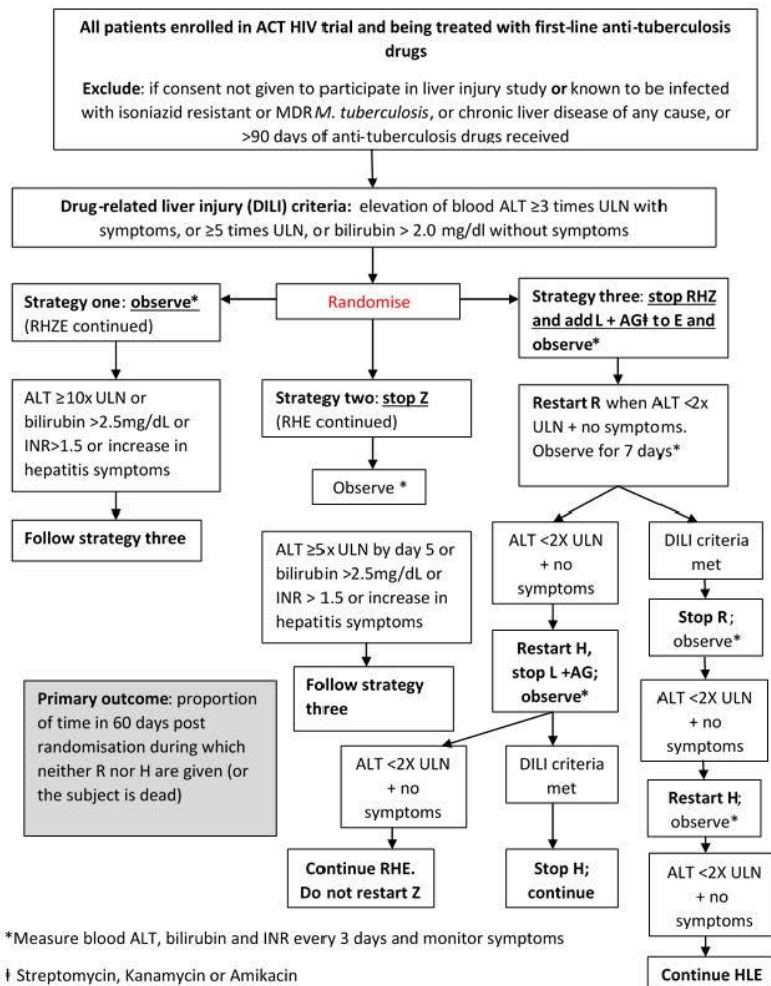


Figure 2. Trial Schema for DILI strategy study.

Ancillary studies

Seven ancillary studies will be conducted within the trial. The studies are as follows:

Ancillary study 1: A randomised comparison of management strategies in response to DILI (as above). (All patients.)

Ancillary study 2: Host and bacterial genetic determinants of treatment response. We hypothesise that the LTA4H gene, and genes involved in related inflammatory pathways, may influence participant inflammatory state, TBM severity, and treatment response. We will also examine genetic variants associated with the development of DILI. (All patients.)

Ancillary study 3: Impact of dexamethasone on CSF inflammation and gross cerebral pathology (assessed by serial brain MRI). We will investigate how dexamethasone influences the resolution of inflammatory markers in the CSF and the gross pathological consequences of TBM on the brain (hydrocephalus, stroke, and tuberculoma formation). (Hospital for Tropical Diseases (HTD) patients only.)

Ancillary study 4: Influence of diabetes mellitus on presentation and response to treatment. We will investigate whether diabetes mellitus influences clinical presentation and CSF inflammatory phenotype (linking with ancillary study 2), and how it impacts upon treatment outcomes. (All patients)

Ancillary study 5: Influence of *Strongyloides* infection on presentation and response to treatment. We will determine whether *Strongyloides* co-infection alters clinical and/or CSF inflammatory phenotype at TBM presentation (linking with ancillary study 2) and treatment response. (All patients.)

Ancillary study 6: Pathophysiology and treatment of hyponatraemia and raised intracranial pressure. We will investigate the pathophysiology of TBM-associated hyponatraemia, enabling a better understanding of the causes of hyponatraemia, the relationship between plasma sodium and elevated intracranial pressure, and the best management of severe hyponatraemia. (HTD patients only.)

Ancillary study 7: Dexamethasone induced adrenal suppression. We will investigate whether the use of corticosteroids in the doses prescribed in infectious diseases is associated with significant adrenal suppression. (HTD patients only.)

Endpoints

Primary endpoint – main trial

The primary endpoint is death or new neurological event (defined as a fall in Glasgow coma score (GCS) by ≥ 2 points for ≥ 2 days from the highest previously recorded GCS (including baseline) or the onset of any of the following clinical adverse events: cerebellar symptoms, focal neurological signs, or new seizures) during 12 months from randomisation. Survivors

without a new neurological event known to be alive at 12 months will be censored at that time-point and subjects who withdrew or were lost to follow-up before 12 months will be censored at the date they were last known to be alive.

Primary endpoint – DILI strategy study

In the DILI strategy study the primary endpoint is the proportion of time in the 60 days following randomisation during which neither rifampicin nor isoniazid are given (or the subject is dead).

Secondary endpoints – main trial

The secondary endpoints of the main trial are as follows:

- Overall survival during a follow-up period of 12 months. Overall survival is defined as the time from randomisation to death, during a follow-up period of 12 months. Survivors known to be alive at 12 months will be censored at that time-point and subjects who withdrew or were lost to follow-up before 12 months will be censored at the date they were last known to be alive.
- Neurological disability at 12 months. Disability will be assessed by the modified Rankin scale (Table 1) on days 30 and 60 from randomisation, and then monthly until the end of anti-tuberculosis drugs. The main endpoint is the 12-month assessment and subjects who died before 12 months will be treated as having a score of 6 ('Dead').
- Severe (grade 3&4) and serious adverse events. Comparison of the frequency of severe and serious adverse events between treatment groups will form an important part of the study analysis.
- Requirement for 'rescue' corticosteroids. Neurological complications occurring after the start of anti-tuberculosis chemotherapy for TBM are common. The cause varies, but includes hydrocephalus, infarcts, tuberculoma formation and hyponatraemia. If the symptoms are thought to be caused by tuberculomas, doctors may re-start or increase the dose of corticosteroids. In this trial, any re-start or dose increase of corticosteroids during the 12-month follow-up will be defined as 'rescue' corticosteroids.

Secondary endpoints – DILI strategy study

The secondary endpoints of the DILI strategy study are as follows:

- Development of acute liver failure (defined as new onset coagulopathy (International normalised ratio (INR) >1.5 and hepatic encephalopathy) after randomisation.
- ART interrupted due to drug-related injury (applies to patients enrolled from linked ACT HIV trial).
- Time to new neurological event (defined as a fall in GCS of ≥ 2 points for ≥ 48 hours, new focal neurological sign, or new onset of seizures) or death from randomisation until the 12 month follow-up of the main trial.

- d) Overall survival, i.e. time to death from any cause, until the 12 month follow-up of the main trial.
- e) Neurological disability at the 12 month follow-up of the main trial.

Inclusion and exclusion criteria

Inclusion criteria

Study participants for LAST ACT must be adults (aged 18 years or older), HIV-uninfected, with a clinical diagnosis of TBM (≥ 5 days of meningitis symptoms, and CSF abnormalities) and anti-tuberculosis chemotherapy either planned or started by the attending physician. Participants will be considered eligible for enrolment in this trial if they fulfil all the inclusion criteria and none of the exclusion criteria. The inclusion and exclusion criteria apply at the point of consent, just before the patient's blood is sent for LTA4H genotyping. Therefore, it is possible that when the LTA4H genotype result returns and the patient is randomised, the patient will have had >3 days of corticosteroids and/or >6 days of anti-tuberculosis treatment. This is acceptable, although the total duration of corticosteroid and anti-tuberculosis treatment before randomisation must be recorded. The time for an LTA4H result to return is not anticipated to be >3 days (e.g. taken on a Friday afternoon and processed on a Monday morning). As TBM is a serious infection requiring hospitalisation, all eligible participants will be treated in hospital, at least for the initial 3 weeks of their illness.

Study participants for the DILI strategy study must be receiving first-line anti-tuberculosis drugs and fulfil the definition of drug-related liver injury: elevation of blood transaminase concentrations ≥ 3 times the ULN with symptoms and signs of hepatitis (vomiting, abdominal pain, jaundice), or ≥ 5 times the ULN or a rise in serum bilirubin $>2.0\text{mg/dL}$ ($>34\text{ }\mu\text{mol/L}$) without symptoms⁹, and less than 90 days of anti-tuberculosis drugs given.

Exclusion criteria

Exclusion criteria for the main trial are:

- a) An additional brain infection (other than TBM) confirmed or suspected: positive CSF Gram or India Ink stain; positive blood or CSF Cryptococcal antigen test; cerebral toxoplasmosis suspected and attending physician wants to give anti-toxoplasmosis treatment with anti-tuberculosis treatment.
- b) More than 6 consecutive days of two or more drugs active against *M. tuberculosis* immediately before screening.
- c) More than 3 consecutive days of any type of orally or intravenously administered corticosteroid immediately before randomisation.
- d) Dexamethasone considered mandatory for any reason by the attending physician.
- e) Dexamethasone considered to be contraindicated for any reason by the attending physician.
- f) Patient has previously been randomised into the trial for a prior episode of TBM.

- g) Lack of consent from the participant or family member (if the participant is incapacitated by the disease).

Exclusion criteria for the DILI strategy study are:

- a) TBM known to be caused by isoniazid resistant or MDR *M. tuberculosis* or standard first-line anti-tuberculosis drugs unable to be given for any reason other than DILI.
- b) Signs of chronic liver disease of any cause (hepatosplenomegaly, prolonged jaundice, caput medusae, spider angiomas, ascites, oedema).
- c) Lack of consent from the participant or family member (if the participant is incapacitated by the disease).
- d) Elevation of blood transaminase concentrations ≥ 3 times the ULN with symptoms and signs of hepatitis (vomiting, abdominal pain, jaundice), or ≥ 5 times the ULN or a rise in serum bilirubin $>2.0\text{mg/dL}$ ($>34\text{ }\mu\text{mol/L}$) without symptoms, at baseline (day 0).

Recruitment, retention and randomisation

Recruitment and retention

Recruitment activities will only occur in an inpatient hospital setting (Hospital for Tropical Diseases and Pham Ngoc Thach Hospital, Ho Chi Minh City, Vietnam). The target sample size of around 720 participants will be enrolled within an anticipated accrual rate of 4 years. Once discharged from hospital the participants will be contacted by phone to remind them of their next visit. Patients who miss a visit will be contacted by phone for a maximum of three times after which a maximum of three home visits will be conducted. As part of routine clinical care participants with suspected TBM will have an HIV test, a lumbar puncture, and a GeneXpert MTB/RIF test on CSF. When possible, participants will be screened for eligibility on the day their CSF results return and at the time the decision is made to start anti-tuberculosis chemotherapy for suspected/confirmed TBM. For the DILI strategy study, based on enrolment of all consenting and eligible patients, we anticipate a total sample size of 170 participants (around 70 HIV-uninfected participants from the LAST ACT trial and a further 100 patients from the linked ACT HIV trial⁹).

Randomisation

Once a participant or their relative has provided written informed consent to enter the study, 5mls of blood will be taken for rapid LTA4H genotyping. The blood will be transported immediately to the OUCRU laboratory within HTD where daily LTA4H genotyping (except at weekends) will be performed. Randomisation will occur once the result of the LTA4H genotyping is available. LTA4H CC- and CT-genotype participants will be randomised to two parallel groups in a 1:1 ratio, receiving either dexamethasone or placebo for 6-8 weeks (according to disease severity). TT-genotype participants will be treated with open-label dexamethasone for 6-8 weeks (according to disease severity). All patients will receive standard anti-tuberculosis chemotherapy. Randomisation will be stratified by participating hospital/site, LTA4H genotype (CC or CT), and modified MRC disease

severity grade (Supplementary File 1) assessed on day 0 when consent is given.

The randomisation list will be computer-generated based on random permuted blocks with variable block size following Oxford University Clinical Research Unit (OUCRU) standard operating procedures. The OUCRU biostatistician in charge of randomisation list preparation will set up statistical code to generate the randomisation list and transfer it to the Study Pharmacist. The Study Pharmacist will then change the random seed, i.e. the initialisation of the random numbers generator, in the statistical code in order to blind the biostatistician and then run the code to prepare the final randomisation list. The generated randomisation lists will be securely incorporated within the trial database. A reliable manual back-up system will also be available. Randomisation to the three strategies for DILI will be 1:1:1 with stratification by initial randomisation (dexamethasone or placebo).

Blinding, unblinding and treatment discontinuation

Blinding

All participants and investigators will be blinded to the treatment allocation. OUCRU clinical trials unit (CTU) pharmacists will create blinded drug packages (in fully made-up and labelled treatment packs) containing either active drugs or identical placebo sufficient for 6–8 weeks of treatment (dependent on the MRC grade of the participant) according to the prespecified randomisation list, and pre-ship them to the sites. After randomisation, the ward or trial research nurse will take the completed prescription form to the site pharmacy; they will dispense the trial-number specific pack containing the study drug. Unused drug will be returned to the site pharmacy if a participant withdraws from treatment. In ancillary study 3 MRI brain images will be read by an independent neuroradiologist, blinded to the treatment allocation and outcomes of the participant.

Unblinding

If, in the opinion of the local clinician, it is important for good clinical care to unblind treatment the documented request will be discussed with the site Principal Investigator (PI) and Chief Investigator (CI). If it is agreed that knowledge of treatment allocation is essential for the best management of the patient, the unblinding code will be provided by the study pharmacist holding the randomisation list at OUCRU CTU upon documented request from the CI. Generalised clinical deterioration is not sufficient for unblinding, given equipoise about the evidence base supporting the use of dexamethasone regardless of clinical severity. All instances of unblinding will be recorded and reported to the Data Monitoring Committee (DMC) and Trial Steering Committee (TSC).

Protocol treatment discontinuation

An individual participant may stop study drug early for any of the following reasons: Participant no longer believed to have TBM and all anti-tuberculosis treatment stopped; Unacceptable toxicity or adverse event; Intercurrent illness that prevents further treatment; Any change in the participant's condition that justifies the discontinuation of treatment in the treating physician's opinion and after discussion with the site PI; Inadequate compliance with the protocol treatment in the judgement of the treating physician; Withdrawal of consent for treatment by the participant.

Trial management

Interventions

All participants will receive the standard of care anti-tuberculosis drugs according to national guidelines. Each participant will be LTA4H genotyped. The enrolment and randomisation of the participant into the trial will be finalised according to genotype. TT-genotype participants will be treated with anti-tuberculosis drugs and open label dexamethasone (Table 2). LTA4H CC- and CT-genotype participants will be

Table 2. Study drug treatment regimen following randomisation.

	MRC Grade I Daily dexamethasone dose/route	MRC Grades II and III Daily dexamethasone dose/route
Week 1	0.3 mg/kg/24 hrs IV	0.4 mg/kg/24 hrs IV
Week 2	0.2 mg/kg/24 hrs IV	0.3 mg/kg/24 hrs IV
Week 3	0.1 mg/kg/24 hrs IV	0.2 mg/kg/24 hrs IV
Week 4	3mg/24 hrs oral	0.1 mg/kg/24 hrs IV
Week 5	2mg/24 hrs oral	4 mg/24 hrs oral
Week 6	1 mg/24 hrs oral	3 mg/24 hrs oral
Week 7	Stop	2 mg/24 hrs oral
Week 8		1 mg/24 hrs oral

randomised to receive dexamethasone or placebo for 6–8 weeks, in addition to anti-tuberculosis drugs, dependent upon disease severity (Table 2). Study drug will be dispensed at randomisation from the site pharmacy in intravenous and oral (tablet) formulations. Placebo will be identical in appearance to active drug and dosed and dispensed in the same way.

Participants for the DILI strategy study will be randomised to one of three strategies (Figure 2); Strategies are as follows:

- 1) Observe: measure transaminases, bilirubin, and INR every 3 days; do not change/stop anti-tuberculosis drugs unless transaminases rise to $\geq 10\times$ normal, or total bilirubin rises $>2.5\text{mg/dl}$ ($>43\text{ }\mu\text{mol/L}$), or INR >1.5 or symptoms of hepatitis worsen (nausea, vomiting, abdominal pain), in which case go to Strategy 3.
- 2) Stop pyrazinamide alone. Observe, measuring transaminases, bilirubin, and INR every 3 days. If transaminases do not fall to $<5\times$ ULN by day 5, or total bilirubin rises $>2.5\text{mg/dl}$ ($>43\text{ }\mu\text{mol/L}$), or INR >1.5 or symptoms of hepatitis worsen at any time (nausea, vomiting, abdominal pain), go to Strategy 3.
- 3) Current standard of care (current US CDC guidelines⁹, Supplementary File 2).

Anti-tuberculosis treatment

First line anti-tuberculosis treatment will follow current Vietnamese national guidelines. Rifampicin (10mg/kg/24 hrs; maximum 600mg), isoniazid (5mg/kg/24hrs; maximum 300mg), pyrazinamide (25mg/kg/24hrs; maximum 2g) and ethambutol (20mg/kg/24 hrs; maximum 1.2g) will be given for at least the first 2 months of treatment, provided drug resistance is not suspected or proven. Pyrazinamide will then be stopped and rifampicin, isoniazid and ethambutol (at the same doses) will then be given until at least 12 months anti-tuberculosis treatment in total has been given. The best treatment of TBM caused by isoniazid-resistant tuberculosis is uncertain, and the attending physician should decide which option to take. For participants with MDR tuberculosis, second-line treatment should be given as soon as possible, following national guidelines and local policies.

Participants with abnormal liver function tests (LFTs) at screening are eligible to enter the trial. These patients should be given standard first-line anti-tuberculosis treatment unless blood transaminase concentrations are ≥ 3 times the ULN with symptoms and signs of hepatitis (vomiting, abdominal pain, jaundice), or ≥ 5 times the ULN or a rise in serum bilirubin $>2.0\text{mg/dL}$ ($>34\text{ }\mu\text{mol/L}$) without symptoms. In these participants, initial anti-tuberculosis treatment should consist of levofloxacin, ethambutol, and an aminoglycoside (either kanamycin, amikacin or streptomycin). LFTs should be monitored every 3 days and rifampicin started as soon as blood transaminases are $<5\times$ the ULN and/or the symptoms and signs of hepatitis resolve. Once the participant is tolerating a rifampicin-containing regimen isoniazid can be introduced and, if isoniazid is tolerated, the aminoglycoside can be stopped. If pyrazinamide is not used in

treatment, at least 12 months of anti-tuberculosis treatment must be given. Participants with liver dysfunction at the start of treatment that require modified initial anti-tuberculosis treatment regimens are ineligible for the DILI strategy study.

Management of hepatitis B

Treating participants infected with hepatitis B (HBsAg positive) with corticosteroids carries a very small risk of reactivation and flare of hepatitis B infection^{10,11}. In order to ensure the safety of patients we will:

- 1) Test all enrolled participants for HBsAg at baseline (day 0).
- 2) After randomisation (of CT- or CC- genotypes) to dexamethasone or placebo, all HBsAg positive participants will be unblinded with respect to the study drug.
- 3) HBsAg positive participants randomised to dexamethasone (CT- or CC- genotypes) will receive tenofovir therapy at 300mg once daily. Tenofovir therapy will be for the duration of dexamethasone therapy, and then for a further 12 months after completion of dexamethasone therapy.
- 4) HBsAg positive participants (of any LTA4H genotype) randomised to placebo will NOT receive tenofovir therapy. Unblinding of HBsAg positive participants is necessary to identify those requiring tenofovir.
- 5) HBsAg positive TT- genotype participants, all of whom will receive open label dexamethasone, will also receive tenofovir therapy at 300mg once daily, according to the current standard of care for HBsAg positive patients as issued by the Vietnam Ministry of Health. Tenofovir therapy will be for the duration of dexamethasone therapy, and then for a further 12 months after completion of dexamethasone therapy.
- 6) LFTs and hepatitis B viral load testing will be performed in those participants with hepatitis B who are receiving dexamethasone. LFTs will be performed at least weekly until discharge, and then every 3 months for the full duration of the study. Liver function tests will be performed more frequently if there is a clinical need. Hepatitis B viral load testing will be performed every 3–6 months (following national guidelines) for the full duration of the study. Participants who are HBsAg negative, but HBcAb positive (i.e. evidence of past hepatitis B infection) will not receive tenofovir and will therefore not be unblinded, but they will be followed carefully for evidence of episodes of hepatitis. The monitoring of HBcAb positive participants (LFTs and hepatitis B viral load testing) will be as for HBsAg patients.

Management of hepatitis C

Participants with hepatitis C infection (positive hepatitis C antibodies) will not be unblinded. However, they will be carefully monitored with liver function tests performed at least weekly

until discharge, and then every 3–6 months for the full duration of the study. Liver function tests will be performed more frequently if there is a clinical need.

Use of concomitant medication

All other concomitant medications essential for participant management are permitted at enrolment, subject to the exclusion criteria of no contraindications to the use of dexamethasone in the judgement of the attending clinician. If use of a concomitant medication that cannot safely be used with dexamethasone becomes essential after randomisation, then the study drug should be stopped and the concomitant medication used without unblinding. Drugs which increase the risk of gastrointestinal bleeding, such as non-steroidal anti-inflammatory drugs (NSAIDs), should be used with caution. Any other oral or intravenously administered corticosteroids are not permitted, unless deemed essential, in which case the study drug should be stopped and replaced by the chosen corticosteroid. Normal standards of clinical care should be followed.

Management of neurological complications occurring after the start of anti-tuberculosis treatment

Corticosteroid use is not routine for the treatment of hydrocephalus or stroke, however for neurological deterioration secondary to tuberculomas most physicians recommend using corticosteroids. In this situation open-label dexamethasone is recommended. High-dose intravenous dexamethasone (0.4mg/kg/24hrs) should be prescribed, and the study drug stopped (if the study drug course is still ongoing).

Data collection

The trial assessment schedule for the main trial is outlined in [Table 3](#).

Clinical assessment

Clinical assessment will include conscious level by GCS, new or ongoing focal neurological deficit, clinical treatment response, all serious adverse events, all adverse events of any grade leading to modification of anti-tuberculosis treatment or its interruption/early discontinuation (and clinician-assessed likelihood of relationship of adverse event to dexamethasone), and adherence to drugs (study drug and anti-tuberculosis drugs). Assessment of disability by the modified Rankin scale will be performed at day 30 from randomisation, monthly until completion of anti-TB drugs, and at month 12.

Inpatient assessment

GCS and new focal neurology will be recorded daily during the participant's hospital admission. Participants will be visited by one of the research team at screening, baseline, randomisation (when LTA4H genotype returns), and at least every 3 days for the first 4 weeks of treatment (unless they are discharged or die before 4 weeks) and then at least every 7 days whilst they remain in hospital. Formal trial clinical assessments will occur on day 0, 3, 7, 10, 14, and weekly thereafter until discharge (+/- 1 day).

Outpatient assessment

After discharge clinical assessments will occur monthly until 12 months. Some of these assessments can be made by phone. Formal outpatient review will occur monthly (+/- 7 days) for at least the first 2 months following hospital discharge. The patient should have formal outpatient review at least every 2 months until month 12 after randomisation. The endpoints of survival and neurological disability by 12 months (-0/+1 month) should be assessed by formal outpatient review whenever possible.

Liver function

Alanine transaminase (ALT) and bilirubin will be measured to evaluate liver toxicity every 7 days until discharge and at each subsequent follow-up visit until anti-tuberculosis treatment stops.

Additional blood tests

EDTA blood will be taken for HBsAg, HBcAb, hepatitis B viral load, and hepatitis C antibodies. Serum will be stored for later DNA extraction when consent has been given.

Glycosylated haemoglobin (HbA1c) and fasting blood sugar

HbA1c and fasting blood sugar will be measured at baseline and at 60 days from randomisation. To enable more detailed phenotyping of diabetes we will also measure C-peptide and blood lipids at baseline and store serum for future diabetes related auto-antibody testing.

Strongyloides

All enrolled participants will be tested for serological evidence of *Strongyloides* infection at baseline (past or latent infection), and with stool examination for evidence of active infection at baseline and at 21–30 days from randomisation, depending on discharge date. When infection is detected, treatment with ivermectin will be provided. Stool will be examined for *Strongyloides* larvae at the end of study drug to determine whether reactivation alters TBM treatment responses.

Synacthen test

We will compare adrenal responsiveness at 3 weeks after randomisation and at the end of study drug treatment (6 or 8 weeks depending upon disease severity) in 100 consecutive patients using the short Synacthen test. The patient's background cortisol level is measured by drawing 2mls of blood at 0900hrs. 250mcg of Synacthen is then administered intravenously; 3ml samples of blood are taken at thirty minutes and sixty minutes to measure the cortisol level after this adrenal stimulation. The Synacthen test will be repeated at day 60 after randomisation.

Lumbar puncture

Lumbar puncture should be performed, unless clinically contraindicated, as part of routine clinical care for the baseline assessment, and on days 30 and 60 after randomisation to assess treatment response. Opening pressure should be measured,

Table 3. Trial assessment schedule.

	SCREENING	DAYS				MONTHS				
		0	RANDOM- ISATION	3	7	WEEKLY UNTIL DISCHARGE	30	60	MONTHLY TO ANTI- TB DRUG END	12
ALL PARTICIPANTS										
Eligibility assessment	(X)									
Participant information sheet and consent	X									
Informed consent		X								
LTA4H genotype (5mls blood)		X								
Clinical assessment	(X)	X	X	X	X	X	X	X	X	X
Disability assessment							X	X	X	X
Chest X-ray	(X)							X		
Lumbar puncture (with paired plasma glucose)	(X)						X	X		
HIV test	(X)									
EDTA blood for genetic tests		X								
Full blood count	(X)				(X)					
Storage for later DNA extraction		X								
Sodium	(X)					(X)			(X)	
Urea/Creatinine	(X)					(X)				
ALT/ bilirubin	(X)				(X)	(X)			(X)	
Hepatitis C antibodies		X								
Hepatitis B surface antigen		X								
Fasting blood sugar/HbA1c		X						X		
C-peptide/Lipids		X								
Strongyloides serology		X								
Serum Storage		X						X		
Stool for Ova, cysts and parasites (Strongyloides) microscopy		X					X			
SUBSET OF PARTICIPANTS RECRUITED TO IMAGING STUDY (HTD only)										
Brain MRI		X						X		X
SUBSET OF PARTICIPANTS RECRUITED TO HYPONATRAEMIA/ICP SUB-STUDY (HTD only)										
24-hour fluid balance	(X)	X		X	X	X				
Plasma sodium		X		X	X	X				
Plasma osmolality										
Urinary sodium		X		X	X	X				
Urinary osmolality										
Plasma cortisol		X								
Doppler Ultra-sound assessment of intravascular volume		X		X	X	X				
Ultra-sound measurement of optic nerve sheath diameter		X		X	X	X				
SUBSET OF PARTICIPANTS RECRUITED TO ADRENAL SUPPRESSION SUB-STUDY (HTD only)										
Synacthen test						X (day 21)		X		

at least 5mls of CSF should be taken for mycobacterial investigations alone, and assessments of cell count and differential, protein, glucose, and lactate should also be performed on 1–2 mls of additional CSF. Ziehl Neelsen stain, GeneXpert, and *M. tuberculosis* culture will be performed on all CSF taken. As part of an ancillary study of the impact of dexamethasone on CSF inflammation and gross cerebral pathology we will measure concentrations of a variety of inflammatory mediators in the CSF (leucocytes, cytokines, chemokines, and eicosanoids, for example) at baseline and on days 30 and 60 after randomisation to determine how dexamethasone influences their expression.

Hyponatraemia and raised intracranial pressure

The rapid diagnosis of intracranial hypertension is challenging and urgently required in TBM. In the subset of participants enrolled to HTD, Ho Chi Minh City, Vietnam, we will investigate the pathophysiology of TBM-associated hyponatraemia by serial assessments of fluid balance, paired plasma and urinary sodium and osmolality, and intravascular volume by Doppler ultrasound assessment of inferior vena cava collapsibility index^{12,13}. We will also use portable ultrasound to measure the optic nerve sheath diameter, which has been shown to be a reliable and non-invasive measure of raised intracranial pressure^{14,15}. These additional measurements will be assessed at days 0, 3, and 7, and weekly until discharge. Plasma cortisol will be measured at day 0.

Imaging

Chest X-ray will be performed at screening and on day 60 after randomisation. Brain imaging by MRI (or CT if the participant cannot tolerate an MRI) will be performed at baseline (+/- 7 days), 60 days (+/- 7 days), and at 12 months (-0/+1 month). We will investigate whether dexamethasone influences the incidence and outcome of hydrocephalus, infarcts and tuberculoma formation in these participants.

Adverse events and safety reporting

Adverse events

Specific procedures will be followed when notifying and reporting adverse events (AEs) or adverse reactions (ARs). The definitions of the EU Directive 2001/20/EC Article 2 based on the principles of ICH good clinical practice (GCP) apply to this trial protocol. All AEs and ARs will be assessed as to whether they are serious or not. If the event is serious and not only related to TBM, or is fatal, then a serious adverse event (SAE) form must be completed and the OUCRU CTU notified within 24 hours. All AEs and ARs (serious and non-serious) should be graded using toxicity gradings.

Causality of all SAEs or serious adverse reactions (SARs) in relation to the trial therapy (dexamethasone) will be assessed. There are five categories: unrelated, unlikely, possible, probable, and definitely, related. If the causality assessment is unrelated or unlikely to be related, the event is classified as an SAE. If the causality is assessed as possible, probable or definitely related, then the event is classified as an SAR. If there is at least a possible involvement of the trial treatment (or comparator), the investigator must assess the expectedness of the event. An unexpected adverse reaction is one not previously reported in the

current Summary of Product Characteristics (SPC) at the time the event occurred, or one that is more frequent or more severe than previously reported. If a SAR is assessed as being unexpected, it becomes a suspected unexpected serious adverse reaction (SUSAR). Investigators should always check the current version of the SPC.

Safety reporting

The OUCRU CTU is responsible for the reporting of SUSARs and other SARs to the regulatory authorities and the research ethics committees. The following events will be reported to the relevant authorities in Vietnam: All unexpected SAEs, all SAEs judged to be related or possibly related to the trial intervention, and all deaths. All SAEs will be reported to OxtREC (Oxford Tropical Research Ethics Committee) in the annual review form and to the DMC in accordance to the DMC charter. An independent DMC will oversee the safety of the trial.

Interim analyses

Interim analyses are planned 6-monthly during the first two years of recruitment and yearly thereafter until the completion of the trial but the DMC has the authority to modify the frequency of interim analyses. At these interim analyses, the DMC will receive a report including unblinded summaries of baseline characteristics, the primary endpoint, overall survival, and adverse events by treatment arm as well as conditional power curves for the primary endpoint. Statistical summaries will be provided both for all randomised subjects and for the subgroup of participants with CC and CT-genotype. The DMC may recommend termination or modification of the study in the circumstances that dexamethasone is harmful (placebo superior), or that dexamethasone is beneficial. The Haybittle-Peto boundary, requiring $p < 0.001$ at interim analysis to consider stopping for efficacy, should be used as guidance in both directions. This boundary may apply to the overall population with CC or CT genotype combined or either genotype alone. Importantly, a DMC recommendation will not be based purely on statistical tables, but will also require clinical judgment. The DMC will also review data from those enrolled into the drug-induced liver injury sub-study, in particular the incidence of acute hepatic failure in each of the management strategy arms.

Statistical analysis

Sample size justification – main trial

In a previous TBM trial¹, 73/189 (38.6%) of HIV-negative subjects with CC-genotype and 69/214 (32.2%) with CT-genotype experienced a neurological event or died during the 9 month follow-up period. Only few additional events are expected between months 9 and 12 of follow-up. All subjects in this trial received dexamethasone. In principle, administration of dexamethasone in CC and CT-genotypes would be discouraged if placebo could be shown to be non-inferior to dexamethasone. However, as the benefit of dexamethasone in the TT-genotype is undisputed and personalised administration of dexamethasone in HIV-uninfected subjects would necessitate rapid genotype testing, some evidence of harm of dexamethasone in the CC/CT population (or the CC group alone) is required. Therefore we have opted for a hybrid trial-design approach which assumes

a modest harm of dexamethasone and aims to prove non-inferiority of placebo first but also allows claiming superiority of placebo in case dexamethasone proves to induce substantial harm¹⁶. Moreover, as it is possible that harm of dexamethasone only applies to the CC-genotype, the trial should allow dropping the CT group at an interim analysis but continue randomisation of the CC group. To protect the one-sided overall familywise error rate of 2.5% for the analysis of the primary endpoint across the two co-primary populations (the full CC/CT population and the CC population), we will assign a multiplicity-corrected one-sided significance level of 2% to the full population and 0.87% to the CC population exploiting the correlation between test statistics on the two populations using the Spiessens and Debois method¹⁷.

We set the non-inferiority margin in favour of dexamethasone at a hazard ratio (HR) of 0.75 and assume a true HR of 1.15 in the CC/CT population. Assuming an absolute risk of a neurological event or death in the dexamethasone group by 12 months of 35%, a HR=1.15 corresponds to risk of 31.2% on placebo, and the non-inferiority margin implies that we can exclude an absolute risk increase of placebo of (at worst) +8.7%. Under these assumptions a total of 184 neurological events or deaths would be required to obtain 80% power at the one-sided 2% significance level. Assuming an overall event risk of $\geq 32\%$, and an 11% sample size increase to compensate for loss-to-follow-up and reductions in power due to the allowance for stopping due to futility, a total of 640 HIV-negative subjects with CC or CT-genotype will be randomised into the trial. Based on experience from our earlier TBM trials^{4,7,18,19}, only a very low number of subjects will be excluded from the per-protocol population, hence only minimally reducing power in this population. Of note, with 184 neurological events or deaths (half of them in the CC group), the trial also has 80% power to prove harm of dexamethasone in the CC/CT group or the CC group in case the true HR is at least 1.54 in all subjects or 1.96 in CC patients, respectively. Our recent trial enrolled 468 HIV-uninfected adults with TBM in 30 months and 90% of them had CC or CT-genotype. Assuming similar recruitment rates, enrolment will be complete within 4 years.

Sample size justification – DILI strategy study

A review of 36 subjects who interrupted rifampicin and isoniazid because of clinical hepatitis or jaundice events from our previous trial⁴ gave the following data: the median (interquartile range (IQR)) onset date of the drug-related liver injury was 50 (15–84) days from initiation of anti-tuberculosis treatment, the median (IQR) duration of the rifampicin and isoniazid interruption was 16 (12–24) days, and 12 subjects subsequently died (8 of them within 60 days). Of note, the duration of the treatment interruption was <30 days for 32 (89%) of the 36 subjects and the remaining 4 subjects never re-started rifampicin or isoniazid but continued to receive alternative anti-tuberculosis treatment for >100 days.

We hypothesise that strategies 1 and 2, respectively, will result in a relative reduction in the duration of the treatment interruption of 50% for subjects with interruptions <30 days, but that they do not affect longer interruptions (as the corresponding

subject might have permanent intolerance to rifampicin and isoniazid) or mortality. Based on simulations of hypothetical trials using re-sampling from the data described above, the hypothesised treatment effect, and the Wilcoxon rank sum test for analysis, we determined that the power to detect such an effect size with a sample size of at least 50 subjects per arm is $>85\%$. Of note, given this is an ancillary and essentially ‘opportunistic’ study, we have chosen a liberal (i.e. not multiplicity corrected) two-sided significance level of 5% for each of the two primary comparisons of strategies 1 and 2, respectively, versus strategy 3.

Analysis populations – main trial

As the effect of dexamethasone may depend on the genotype with worse anticipated outcomes for CC-genotype, this trial has two co-primary analysis populations: The full analysis population containing all randomised patients and the subgroup of subjects with CC-genotype. Analyses in the subgroup with CT-genotype will also be performed but are exploratory only. In addition, the primary end point will be analysed in the per-protocol population, which will exclude the following patients: patients with a final diagnosis other than TBM, major protocol violations and those receiving less than 1 week of administration of the randomised study drug for reasons other than death. In all populations, patients will be analysed according to their randomised arm. For the analysis of the primary endpoint, a multiplicity corrected one-sided significance level of 2% for the full population and 0.87% for the CC subgroup will be applied as described in the sample size justification above. For all other analyses, the conventional two-sided significance level of 5% will be applied. Published diagnostic criteria²⁰ will be applied to all enrolled participants at the end of the study when all mycobacterial culture results are available (Supplementary File 3). The criteria will sub-divide all cases into definite, probable and possible TBM, and those with an alternative diagnosis.

For the primary analyses of the main trial the second randomisation in the DILI strategy study will be ignored and the estimated dexamethasone treatment effect can thus be interpreted as an average effect across the three management strategies. We believe that this is justified because only approximately 70 (11%) subjects are expected to be enrolled in the nested trial with roughly similar numbers from both arms, because the efficacy of the different management strategies is unlikely to depend on whether the patient received dexamethasone or not as it tests a very different intervention, and because the anticipated effect of the management strategy on neurological events and deaths is relatively small. However, in a supplementary analysis, we will also compare the primary endpoint between the treatment policies “dexamethasone treatment plus standard of care management of drug-related liver injury” vs. “placebo treatment plus standard of care management of drug-related liver injury” using an inverse probability weighting based analytical framework²¹.

Analysis populations – DILI strategy study

All patients in the DILI strategy study will be analysed according to their randomised arm as an intention-to-treat (ITT) analysis. The two primary comparisons are the comparisons of

strategies 1 and 2, respectively, versus strategy 3 (with tests conducted at the unadjusted two-sided 5% significance level) and comparisons between strategies 1 and 2 will be exploratory only.

Primary endpoint analysis – main trial

The primary endpoint of this trial is the time to death or a new neurological event during 12 months of follow-up. The primary endpoint will be analysed using a Cox proportional hazards regression model with treatment as the only covariate and stratification by TBM MRC severity grade at enrolment (I, II, or III) and LTA4H genotype (CC or CT). The primary effect measure is the resulting hazard ratio comparing dexamethasone vs. placebo with a corresponding confidence interval and p-value. Non-inferiority of placebo in the entire population or the CC-genotype subgroup will be established if the corresponding test (at the one-sided 2% or 0.87% significance level for the full population and the CC subgroup, respectively) rejects the null hypothesis that dexamethasone decreases the hazard of the primary endpoint by 25% or more. Superiority of placebo will additionally be established, if the null hypothesis that dexamethasone does not affect the hazard of the primary endpoint can be rejected against the one-sided alternative that dexamethasone causes harm. The distribution of the primary endpoint will also be visualized using Kaplan-Meier plots and explicit survival estimates at 3, 6, 9, and 12 months of follow-up will also be calculated.

The proportional hazards assumption will be formally tested based on scaled Schoenfeld residuals and visually assessed by a plot of the scaled Schoenfeld residuals versus transformed time. In case of a significant test, a formal comparison of the absolute risk of an event by 12 months between the two groups will also be performed (using a Wald-type test based on Kaplan-Meier estimates at 12 months and associated standard errors using Greenwood's formula). The homogeneity of the treatment effect on the primary endpoint across subgroups will be assessed by subgroup analyses and formal tests of interactions between treatment and the following grouping variables: genotype (CC or CT), TBM MRC severity grade at enrolment (I, II, or III), and drug resistance pattern (MDR-TB or rifampicin mono-resistance, isoniazid resistant non-MDR, no or other resistance). To obtain an adjusted treatment effect estimate and to assess the effect of other covariates, the primary endpoint will also be modelled using a multivariable Cox proportional hazards regression model including the following covariates (in addition to the treatment group): genotype, TBM MRC severity grade at enrolment, and drug resistance pattern.

Primary endpoint analysis – DILI strategy study

For the analysis of the primary endpoint of the DILI strategy study, the non-parametric Wilcoxon rank sum test will be used for pairwise comparisons. An additional adjusted analysis (with adjustment for the initial randomisation, HIV-status, and the time from initial randomisation to the second randomisation) will be also be performed treating the outcome as an ordinal outcome and using a proportional odds logistic regression model (which can be interpreted as an extension of the Wilcoxon rank sum test).

Secondary endpoint analysis

Overall survival will be analysed in the same way as the primary endpoint (except that non-inferiority will not be formally assessed). Neurological disability (as assessed by the ordinal modified Rankin scale) at 12 months will be compared between the two arms with a proportional odds logistic regression model with the treatment assignment as the main covariate and adjustment for genotype and TBM MRC severity grade. The result will be summarised as a cumulative odds ratio with a corresponding 95% confidence interval and p-value. Patients with a missing 12 month disability assessment will be excluded from the main analysis but an alternative analysis based on multiple imputation (including disability assessments at earlier time points in the imputation model) will also be performed. The number and proportion of subjects requiring 'rescue' corticosteroids will be summarised by treatment arm. Comparisons will be based on logistic regression with the treatment assignment as the main covariate and adjustment for genotype and TBM MRC severity grade.

Analysis of adverse events

The number of patients with any adverse events and specific events, respectively, will be summarised and informally compared between the two treatment arms based on Fisher's exact test. The total number of adverse event episodes per patient will also be summarised and informally compared based on a quasi-Poisson regression model with treatment as the only covariate. The following subgroups of adverse events will also be separately summarised: grade 3&4 adverse events; serious adverse events; serious adverse events possibly, probably, or definitely related to the study drug; adverse events leading to TB treatment interruptions. Grade 3&4 laboratory abnormalities will be summarised in the same way as clinical adverse events.

Baseline descriptive analyses

Baseline characteristics will be summarised as median (lower and upper quartiles) for continuous data and frequency (percentage) for categorical data. The amount of missing data for each baseline characteristic will also be displayed.

Ethical considerations

Confidentiality

Participants' confidentiality will be maintained throughout the trial. Participants will be assigned a trial identification number and this will be used on case report forms (CRFs); participants will not be identified by their name. The investigator will securely keep a participant trial register showing identification numbers, surnames and date of birth. The unique trial number will identify all laboratory specimens, case record forms, and other records and no names will be used, in order to maintain confidentiality. Data submitted to OUCRU CTU and samples sent to central testing facilities will be identified only by the trial number and participant initials.

Consent

Written informed consent must be obtained in order to enter into the trial and be randomised. If a participant lacks capacity,

written consent must be obtained from a person with responsibility (e.g. family member/relative), in their own language before enrolment by the site PI or an appropriately trained doctor. All potential participants (or their families) will be given a participant information sheet clearly listing the risks and benefits of the trial. All potential participants (or their families) will be able to discuss participation with their consulting doctor who will be able to address questions not covered or arising from the participant information sheet. Incapacitated adults with TBM will be eligible to enter the trial. These adults have more severe disease and therefore may benefit most from adjunctive dexamethasone. We anticipate around 70% of participants with TBM will lack capacity at the start of treatment. An option will be given to patients to enrol in the main study, but not the ancillary studies.

If consent is provided by a relative, the participant should be consulted and consent recorded if and when they have the capacity to do so. If they are happy to remain in the trial, the participant should complete a participant consent form at this time. If they wish to withdraw from the trial, no further trial-related procedures will be performed, but data to this point would be used in analysis. Data from any participant who dies before regaining capacity (but whose family member has provided consent) will be included in analysis.

Ethical approval

The trial protocol has been approved by the Oxford Tropical Research Ethics Committee, the Ethics Committees of the Hospital for Tropical Diseases Pham Ngoc Thach Hospital and the Vietnam Ministry of Health.

Protocol violations

All deviations from protocol will be addressed in source documents and reported to the OUCRU CTU.

Withdrawing from the trial

A participant (or their relative) is free to refuse to participate in or withdraw from all or any aspect of the trial, at any time and for any reason. If a participant chooses to discontinue their trial treatment they should always be followed up (providing they are willing) and they should be encouraged not to leave the whole trial. If they do not wish to remain on trial follow-up however, their decision must be respected and the participant will be withdrawn from the trial. Participants may change their minds about stopping trial follow-up at any time and re-consent to participation in the trial.

Data collection and storage

Clinical data and clinical laboratory data will be entered into CliRes, a 21 CFR Part 11-compliant data capture system provided by the OUCRU information technology department. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Trial data will be recorded onto paper CRFs and entered into CliRes. The participants will be identified by a unique trial specific number and/or code in any database. The name and any other identifying detail will

not be included in any trial data electronic file. CRFs, clinical notes and administrative documentation will be kept in a secure location and held for 15 years after the end of the trial. Clinical information will not be released without written permission, except as necessary for monitoring, auditing and inspection purposes. Electronic data will be kept for at least 20 years at the OUCRU CTU.

SPIRIT checklist

A SPIRIT checklist for this trial protocol is attached ([Supplementary File 4](#)).

Trial Committees

A Trial Management Group (TMG) will be formed to conduct the day-to-day management of the trial at the OUCRU CTU. This will include the CI, Head of OUCRU CTU, Trial Statistician, Clinical Project Manager, Trial Manager and Data Manager. The group will meet at least once per month, although may meet more or less often as required. The TSC has membership from the TMG plus independent members (Professor Nicholas Paton (Infectious Diseases physician and Clinical Trialist, National University of Singapore, Singapore), Professor Ben Marais (Senior Tuberculosis Researcher and Trialist, University of Sydney, Australia), DrTruong Huu Khanh (Infectious Diseases Physician, Paediatric Hospital Number 1, Ho Chi Minh City, Vietnam)), including the Chair (Professor Robert Wilkinson (Honorary Professor and Director Wellcome Centre for Infectious Diseases Research in Africa, University of Cape Town, South Africa)). The role of the TSC is to provide overall supervision for the trial and provide advice through its independent chair. The ultimate decision for the continuation of the trial lies with the TSC. The DMC (Professor Sarah Walker (DMC Chair, Senior Statistician and Clinical Trialist, MRC Clinical Trials Unit, University College London), Professor Graeme Meintjes (Senior Infectious Diseases/HIV Physician, University of Cape Town, South Africa) and Professor Nina Ruslami (Senior TBM Clinician and Researcher, Universitas Padjadjaran, Bandung, Indonesia)), will advise the TSC and can recommend premature closure or reporting of the trial, or that recruitment be discontinued or modified. The DMC will advise the TSC and can recommend premature closure or reporting of the trial, or that recruitment be discontinued or modified. The DMC is independent from the sponsor. Access to interim data and results will be confidential and strictly limited to the DMC and results (except for the recommendation) will not be communicated to the outside and/or clinical investigators involved in the trial. This trial is sponsored by The University of Oxford (Contact: University of Oxford, Research Services, University Offices, Wellington Square, Oxford OX1 2JD, Tel +44 (0) 1865 282585).

Data dissemination

Manuscripts arising from the trial will, wherever possible, be submitted to peer-reviewed journals which enable Open Access via UK PubMed Central within six months of the official date of final publication. In line with research transparency and greater access to data from trials OUCRU's clinical trials are registered at ClinicalTrials.gov and a data sharing policy is in

place. Data exchange complies with Information Governance and Data Security Policies in all of the relevant countries.

Discussion

Dexamethasone has been shown to improve survival in HIV-uninfected individuals with TBM¹⁸. Previous data strongly suggests hyperinflammatory LTA4H TT-genotype patients with TBM benefit from dexamethasone, and that adjunctive dexamethasone does not benefit, and may cause harm, when given to patients with LTA4H CT or CC-genotype⁵. How corticosteroids improve survival in TBM in HIV-uninfected patients, and whether they do so in all HIV-uninfected patients, remains uncertain and is the focus of the LAST ACT trial. Adjunctive dexamethasone is currently standard of care for HIV-unaffected patients with TBM. This study may identify a role for using LTA4H genotype to guide adjunctive anti-inflammatory therapy.

Trial status

Trial protocol version 1.6. Estimated recruitment start date 1st February 2018. Estimated time for recruitment is 4 years.

Ethics statement

The trial has ethics approval from the Oxford Tropical Research Ethics Committee (approval number 52-16), the Ethics Committees of the Hospital for Tropical Diseases (approval number CS/ND/17/27) and Pham Ngoc Thach Hospital (approval number CS/PT/17/06), and the Vietnam Ministry of Health.

Data availability

No data are associated with this article.

Disclosures

Due to the linked nature of this trial, some sections of this protocol also form part of the linked RCT ACT HIV (Trial registration number: [NCT03092817](#)), which has also been submitted to *Wellcome Open Research*⁸. ACT HIV is a parallel group, randomised (1:1), double blind, placebo-controlled multi-centre Phase III trial, comparing the effect of dexamethasone versus placebo on overall survival in HIV-infected patients with TBM. The 7 ancillary studies in LAST ACT are also recruited to through the ACT HIV trial. As such the hypotheses, design, methods, sample size justification, analysis plans and endpoints of these ancillary studies will also be described in the ACT HIV trial,

and will appear identically here. LAST ACT follows the same OUCRU protocols and local / national guidelines as ACT HIV, therefore randomisation, blinding and unblinding procedures, adverse event and safety reporting, ethics and confidentiality sections also appear identically here for LAST ACT as for the ACT HIV trial.

Author contributions

JD, writing – original draft preparation, supervision, methodology. NHP, supervision, methodology. LTPT, formal analysis. NHL, supervision, methodology, project administration. NTHM, investigation, methodology. NTMT, supervision, methodology, project administration. NTTH, investigation, project administration. TBN, investigation, project administration. BTBH, investigation, project administration. VTPM, project administration. NDB, supervision, methodology, project administration. DCG, supervision, methodology, project administration. DTMH, supervision, methodology, project administration. JD, methodology. NTHH, methodology. NNV, supervision, investigation. RBG, formal analysis. TTH, supervision, methodology. EK, supervision, methodology, writing – review and editing. MW, formal analysis. NVVC, supervision, methodology. GET, conceptualisation, funding acquisition, supervision, writing – review and editing.

All authors read and approved the final manuscript.

Competing interests

No competing interests were disclosed.

Grant information

The trial is supported by the Wellcome Trust [110179], an Investigator Award to Professor Guy Thwaites.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgements

We would like to acknowledge the important contributions of all doctors nurses and patients who will make the trial a success. We would like to thank OUCRU CTU administrative staff for their help in conducting the study.

Supplementary material

Supplementary File 1. Modified MRC grade of TBM.

[Click here to access the data.](#)

Supplementary File 2. Current standard of care in DILI.

[Click here to access the data.](#)

Supplementary File 3. TBM diagnostic criteria.

[Click here to access the data.](#)

Supplementary File 4. SPIRIT checklist.

[Click here to access the data.](#)

Supplementary File 5. LAST ACT consent form for HTD Vietnam.

[Click here to access the data.](#)

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Katalin A. Wilkinson 

¹ Wellcome Centre for Infectious Diseases Research in Africa, Institute of Infectious Diseases and Molecular Medicine, University of Cape Town, Observatory, South Africa

² The Francis Crick Institute, London, UK

TB meningitis is the most severe form of extrapulmonary TB and adjunctive dexamethasone is currently standard of care for HIV-uninfected patients. This trial will capitalise on previous data showing that the survival benefit of adjunctive dexamethasone was restricted to the LTAH4 TT-genotype patients with high inflammatory states. Therefore, HIV uninfected TBM patients with LTAH4 CC and CT genotype will be randomised to adjunctive dexamethasone or placebo, to determine the noninferiority of placebo, with the hypothesis that dexamethasone has no benefit in patients with CC or CT genotype. The study design is complex but meticulous, with seven ancillary studies as well as a parallel, identical but independent study run in HIV-infected patients. These studies will be the ultimate proof of host directed therapies in TB.

Questions:

1. Related to ancillary study 1 (management strategies in response to DILI): the numbers anticipated in the HIV-uninfected patients is n~70, to be analysed according to the 3 strategies. This may mean that the numbers in these analyses might be relatively low. Since the DILI strategy study will also be run in the ACT-HIV study, where anticipated numbers are n~100, will the two groups be potentially combined for analysis? It is acknowledged that this will present a number of limitations with respect to drug interactions when considering ART in the HIV-infected patients.
2. Related to ancillary study 2: will genetic variants associated with development of DILI be examined related to the host or bacteria, or both?
3. Related to ancillary study 3: the influence of dexamethasone on the resolution of inflammatory markers in the CSF will be examined. This should be done in parallel with blood, although I see no blood collection for such studies in Table 3.
4. Considering that there is no LTAH4 genotyping at weekends, and a patient recruited on a Friday may have been already on anti-TB treatment for 5 days with adjunctive dexamethasone. Therefore, by the time the genotyping result is returned on Monday with a CC genotype, the patient already received 8 days of potentially harmful dexamethasone. Assuming that this patient is

- randomised to placebo, the harm is potentially already done, therefore having a negative effect on the placebo arm. Is there a way to control for this, by potentially not recruiting on a Friday or introducing weekend genotyping for the duration of the trial.
5. HIV testing is done at baseline: would there be benefit in further HIV testing at 3 and/or 6 months?

Is the rationale for, and objectives of, the study clearly described?

Yes

Is the study design appropriate for the research question?

Yes

Are sufficient details of the methods provided to allow replication by others?

Yes

Are the datasets clearly presented in a useable and accessible format?

Not applicable

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 24 Oct 2018

Joseph Donovan, Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam

Dear Dr Wilkinson,

Many thanks for your comments.

I have included replies to each of your points as below:

1. Related to ancillary study 1 (management strategies in response to DILI): the numbers anticipated in the HIV-uninfected patients is n~70, to be analysed according to the 3 strategies. This may mean that the numbers in these analyses might be relatively low. Since the DILI strategy study will also be run in the ACT-HIV study, where anticipated numbers are n~100, will the two groups be potentially combined for analysis? It is acknowledged that this will present a number of limitations with respect to drug interactions when considering ART in the HIV-infected patients.

Author reply

These two DILI patient groups (from LAST ACT and from ACT HIV) will be analysed together (and a separate analysis will be performed stratified by HIV status). When anti-TB drugs are stopped because of DILI, the use and cessation of other potentially hepatotoxic (and DILI-causing) drugs will also be recorded, including ART.

2. Related to ancillary study 2: will genetic variants associated with development of DILI be examined related to the host or bacteria, or both?

Author reply

Host only

3. Related to ancillary study 3: the influence of dexamethasone on the resolution of inflammatory markers in the CSF will be examined. This should be done in parallel with blood, although I see no blood collection for such studies in Table 3.

Author reply

Only CSF inflammatory markers will be analysed at this time

4. Considering that there is no LTA4H genotyping at weekends, and a patient recruited on a Friday may have been already on anti-TB treatment for 5 days with adjunctive dexamethasone. Therefore, by the time the genotyping result is returned on Monday with a CC genotype, the patient already received 8 days of potentially harmful dexamethasone. Assuming that this patient is randomised to placebo, the harm is potentially already done, therefore having a negative effect on the placebo arm. Is there a way to control for this, by potentially not recruiting on a Friday or introducing weekend genotyping for the duration of the trial.

Author reply

The number of days of dexamethasone received will be recorded, including any days given whilst waiting for the LTA4H result to return. The effect of dexamethasone on individuals randomised to placebo will be factored in to the analysis. Genotyping is now available on one day over the weekend which will reduce this effect.

5. HIV testing is done at baseline: would there be benefit in further HIV testing at 3 and/or 6 months?

Author reply

Additional HIV testing was considered, however a decision was made to only perform this if clinically indicated, rather than as part of the trial.

Competing Interests: None

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Ravindra Kumar Garg

Department of Neurology, King George's Medical University, Lucknow, Uttar Pradesh, India

This is wonderfully planned almost flawless study. I have two questions

1. Denying corticosteroids to a subgroup of patient, will it be ethically appropriate?
2. Among CC or CT- LTA4H genotype or TT- genotypes which one have inherently poor prognosis and need corticosteroids? "LTA4H genotype may be a critical determinant of inflammation and consequently of adjunctive anti-inflammatory treatment response" --- outcome among varied genotypes should have been additional factor determining suitability for corticosteroids.

Is the rationale for, and objectives of, the study clearly described?

Yes

Is the study design appropriate for the research question?

Yes

Are sufficient details of the methods provided to allow replication by others?

Yes

Are the datasets clearly presented in a useable and accessible format?

Yes

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

The neurocritical care of tuberculous meningitis

Joseph Donovan, Anthony Figaji, Dama Imran, Nguyen Hoan Phu, Ursula Rohlwink, Guy E Thwaites



Tuberculous meningitis is the most severe form of tuberculosis and often causes critical illness with high mortality. Two primary management objectives are reducing intracranial pressure, and optimising cerebral perfusion, while killing the bacteria and controlling intracerebral inflammation. However, the evidence base guiding the care of critically ill patients with tuberculous meningitis is poor and many patients do not have access to neurocritical care units. Invasive intracranial pressure monitoring is often unavailable and although new non-invasive monitoring techniques show promise, further evidence for their use is required. Optimal management regimens of neurological complications (eg, hydrocephalus and paradoxical reactions) and of hyponatraemia, which frequently accompanies tuberculous meningitis, remain to be elucidated. Advances in the field of tuberculous meningitis predominantly focus on diagnosis, inflammatory processes, and antituberculosis chemotherapy. However, clinical trials are required to provide robust evidence guiding the most effective supportive, therapeutic, and neurosurgical interventions for tuberculous meningitis that will improve morbidity and mortality.

Introduction

Mycobacterium tuberculosis is responsible for approximately 10 million new cases of tuberculosis and 1–3 million deaths annually.¹ Tuberculous meningitis is the most severe form of the disease, killing or severely disabling around 50% of affected patients (panel 1).² Tuberculous meningitis disproportionately affects children and those with HIV infection.³ Clinical onset of tuberculous meningitis is indolent and diagnosis is challenging.⁴ Treatment of the disease is lengthy and optimal drug regimens are uncertain. Thick obstructing intracerebral exudates and inflammatory lesions result in raised intracranial pressure, which leads to clinical neurological deterioration, coma, and death.

Advances in the field of tuberculous meningitis include standardisation of tuberculous meningitis clinical research methods (such as collection of datasets and outcome reporting),⁵ improved diagnosis,⁶ increased understanding of host genetic influence on intracerebral inflammation and survival,^{5,11} and identification of first-line antituberculosis treatment regimens.^{10,15} Yet, critical illness caused by tuberculous meningitis is an area that requires further research. No guidelines exist for the management of critical illness associated with tuberculous meningitis, and the evidence base for treatment is poor. Studies of critically ill patients with tuberculous meningitis are largely retrospective, observational, and contain small patient numbers,^{16–19} with paediatric data particularly scarce.²⁰ Patient outcomes in those admitted to critical care units are extremely poor, with studies predominantly involving adults showing 40–53% mortality, and neurological disability is common in survivors.^{16,19} Severe tuberculous meningitis presents specific clinical challenges, in particular the detection and management of raised intracranial pressure and brain ischaemia.⁷ Further research to optimise supportive care and neurosurgical interventions is required to improve functional outcomes of patients with tuberculous meningitis.²¹

In this Review, we focus on aspects relevant to the neurocritical care of patients with tuberculous meningitis, with general diagnosis and treatment reviewed elsewhere.⁷

We start by discussing causes of critical illness, focusing on causes that occur earliest and those which contribute most to mortality and morbidity. We then describe complications in disease progression, including those arising from treatment. We then discuss supportive management, followed by medical, and then surgical management. We conclude by suggesting future research directions and proposing clinical trials that are required to improve outcomes from critical illness arising from the disease.

Causes of critical illness in tuberculous meningitis

Raised intracranial pressure

Coma in tuberculous meningitis is associated with raised intracranial pressure.⁷ A sustained intracranial pressure of more than 20 mmHg is considered abnormal in adults,²² although pressure changes have not been correlated with prognosis in patients with tuberculous meningitis and the true incidence of raised intracranial pressure in patients with tuberculous meningitis is uncertain. The reduction of cerebral blood flow in conditions of raised intracranial pressure, after limits of compensatory changes are reached, is an unproven mechanism in the disease. The normal intracranial pressure range in children is lower than in adults and depends on age.²³ Hydrocephalus results from CSF blockage at either the basal cistern or absorptive arachnoid granulations (so-called communicating hydrocephalus), or at the cerebral aqueduct or fourth ventricle outlet (non-communicating hydrocephalus).^{8,24} and is the most common cause of raised intracranial pressure in patients with tuberculous meningitis.⁷ In two studies of adolescents (older than age 14 years) and adults with tuberculous meningitis in India, 109 (52%) of 209 patients and 52 (65%) of 80 patients had baseline MRI images consistent with hydrocephalus.^{25,26} Most patients (70–80%) have communicating hydrocephalus.³ The two types of hydrocephalus increase intracranial pressure and manifest clinically with headache, reduced consciousness or focal neurological deficits, or both. Cerebral oedema⁸ and tuberculoma formation²⁷ could also elevate intracranial pressure in patients with tuberculous

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Panel 1: Pathophysiology of tuberculous meningitis

Tuberculous meningitis results from the haematogenous dissemination of *Mycobacterium tuberculosis* to the brain, followed by granuloma rupture and bacterial inoculation into the subarachnoid space.² An insidious onset of non-specific prodromal symptoms followed by a slowly progressive meningitis makes diagnosis challenging, with a wide range of differential diagnosis, especially in patients with HIV co-infection.³ Delayed diagnosis and treatment lead to poor outcomes,⁴ and inflammatory pathways are not completely understood. In patients with HIV co-infection, mortality is associated with higher CSF neutrophil counts,⁵ yet how neutrophils drive inflammation in tuberculous meningitis is unclear.⁶ Intracerebral inflammation is thought to contribute to the poor prognosis associated with tuberculous meningitis.¹ Inflammatory pathways and adjunctive anti-inflammatory therapies have been reviewed elsewhere.⁷

meningitis. Severe ischaemic strokes can cause brain shift and raise intracranial pressure,²⁸ although such events are not well described for patients with tuberculous meningitis. Seizures,²⁹ fever, impaired ventilation, and hyponatraemia, which are symptoms frequently observed in tuberculous meningitis, might raise intracranial pressure to a critical level, resulting in brain injury.⁷

Cerebral infarction

Cerebral infarction is the main cause of long-term neurological disability in patients with tuberculous meningitis.⁷ The exact pathophysiology of cerebral infarction associated with tuberculous meningitis is uncertain, although inflammation and necrosis secondary to surrounding basal exudate are thought to contribute to blood vessel pathology.^{8,30} The lateral and medial lenticulostriate arteries are most commonly affected,³¹ with resulting basal ganglia infarcts, although subcortical white matter infarcts have also been reported in patients with tuberculous meningitis.³² Vasculitis is common, vasospasm is uncommon, and complete vessel occlusion of major arteries is unusual, but has been reported in patients with tuberculous meningitis.^{8,32} Two MRI studies in adults with tuberculous meningitis showed cerebral infarction in 40 (35%) of 114 patients³⁰ and 34 (67%) of 51 patients.³¹ A systematic review of six studies including 843 children with tuberculous meningitis showed that cerebral infarction was present in 255 children at admission.⁹ Impaired cerebral perfusion leads to ischaemia, cerebral infarction, and raised intracranial pressure.⁷ Hemiplegia is the most common clinical consequence of cerebral infarction due to tuberculous meningitis.⁸ The range of neurological complications resulting from cerebral infarction associated with tuberculous meningitis has not been studied in detail over the past 10 years, and the effect of irreversible neurological disability on feeding, pneumonia, pressure damage, and thrombosis remains unknown.

Tuberculoma

A tuberculoma is the result of granulomatous inflammation forming space-occupying brain lesions, after metastatic seeding of *M. tuberculosis* to the CNS.²⁷ Tuberculomas are the most common cause of paradoxical reactions in HIV-negative patients with tuberculous meningitis.³³ A systematic review⁹ of seven studies including 1056 children with tuberculous meningitis showed that tuberculomas were present in 112 children at admission, compared with 15 (13%) of 114 HIV negative adults with tuberculous meningitis in a randomised controlled trial (RCT),³⁰ indicating that they play a role in both paediatric and adult cases of tuberculous meningitis.³³ Tuberculomas might exert local mass effect on brain tissue, causing compression of cerebral ventricles, leading to headache, vomiting, decreased consciousness, focal neurological signs, and seizures.²⁷ Tuberculomas can have a severe effect on outcome and might cause severe disability.

Hyponatraemia

Hyponatraemia can develop at any time during treatment of tuberculous meningitis. Hyponatraemia is classified as profound when sodium falls below 125 mmol/L.³⁴ In one study of adults and children with tuberculous meningitis, 34 (45%) of 76 individuals had hyponatraemia, and 8 (11%) patients had sodium values below 120 mmol/L.³⁵ Cerebral salt wasting and the syndrome of inappropriate antidiuretic hormone secretion are considered the most likely causes of hyponatraemia in patients with tuberculous meningitis and could overlap;³⁶ yet the mechanism of hyponatraemia remains poorly understood. In cerebral salt wasting, renal sodium loss could be mediated by natriuretic peptides, whereas in the syndrome of inappropriate antidiuretic hormone secretion, vasopressin secretion occurs independently of serum osmolality or circulating volume.³⁴ Diagnosis is confounded by varying definitions of cerebral salt wasting and the syndrome of inappropriate antidiuretic hormone secretion and similarity of electrolytes and osmolality values.³⁵ Hyponatraemia occurs with brain injury³⁶ and contributes to raised intracranial pressure. Hyponatraemia worsens cerebral oedema, with acute sodium changes particularly harmful for patients.³⁴ Hyponatraemia causes headache and confusion (seizures and coma when severe)³⁴ and predicts increased mortality in patients with HIV and tuberculous meningitis.³⁷

Paradoxical reactions

Paradoxical reactions are when worsening signs and symptoms of tuberculosis occur despite effective anti-tuberculosis chemotherapy.³⁸ These reactions are commonly considered an exuberant inflammatory response to dead or dying bacteria; their pathogenesis is poorly understood.³⁸ In a study of patients with tuberculous meningitis in India (mean age 30 years), 44 (31%) of 141 patients developed a paradoxical reaction.³⁹ These

reactions usually occur after 2–4 months of antituberculosis chemotherapy.³³ Paradoxical reactions contribute to critical illness of patients with tuberculous meningitis, with clinical features including headache, altered vision, and seizures.³⁹ Neuroimaging findings include enhancing basal exudates, new or worsening tuberculomas, and optochiasmatic or spinal arachnoiditis.³⁹ Paradoxical changes might cause mass effect and obstruct CSF,³⁹ raising intracranial pressure. Clinical spinal disease is common and might be overlooked and is often revealed after commencing antituberculous chemotherapy. High CSF protein concentrations indicate a greater likelihood of development.⁴⁰ Lumbosacral disease is most common,⁴⁰ and urinary retention is often the first presenting symptom.

Drugs

Critical illness can result from or be exacerbated by side-effects of antituberculosis chemotherapy and interactions with other drugs (table 1). Drug-induced liver injury is the most common drug-associated adverse event of antituberculosis chemotherapy,³¹ with severe symptoms such as vomiting and abdominal pain. Drug reactions affecting neurological status might confound monitoring of patients with tuberculous meningitis. Antituberculosis chemotherapy such as fluoroquinolones^{35,46} and isoniazid⁴⁷ can increase seizure risk, and both isoniazid and fluoroquinolone treatments might cause psychiatric disorders.

Antituberculosis drug resistance

Tuberculosis caused by *M tuberculosis* that is resistant to first-line antituberculosis drugs is becoming increasingly common worldwide³ and increases the risk of treatment failure and death. Tuberculous meningitis caused by bacteria resistant to at least rifampicin and isoniazid (ie, multi-drug resistance) is mostly fatal unless second-line drugs are administered early.⁴⁷ Prevalence of multidrug resistance in tuberculous meningitis is about 4% in Europe⁴⁸ and 12% in China.⁴⁹ Mono-isoniazid resistance is more common (nine [6%] of 142 cases in the European study⁴⁸) and is associated with worse outcomes, although its effect on treatment response is less than that of multidrug resistance.⁴⁷ The low sensitivity of current molecular diagnostic tests, which can detect *M tuberculosis* and drug resistance (eg, GeneXpert) in CSF, and the 4–8 weeks taken to obtain in-vitro drug susceptibility testing information, means that determining whether drug resistance is causing or contributing to critical illness caused by tuberculous meningitis is extremely challenging.

Young children and individuals with HIV co-infection

Specific risks apply to young children and individuals with HIV. Intracerebral inflammation is increased in individuals with HIV and tuberculous meningitis.³¹ Immune reconstitution inflammatory syndrome (IRIS) might develop, presenting as new or worsening neurological symptoms after the introduction of antiretroviral

therapy.⁵⁰ Neurological manifestations of IRIS include meningitis, brain tuberculoma, brain abscess, radiculomyelitis, and spinal epidural abscess.⁵¹ The effect of HIV co-infection on the pharmacokinetics of first-line antituberculosis chemotherapy is uncertain; a systematic review of 27 studies was unable to conclude a clear effect.⁵² Antiretroviral therapy is complicated by rifampicin's induction of cytochrome P450 enzymes,⁵³ and drug-induced liver injury could be more common in patients with HIV. Miliary tuberculosis⁵⁴ (in which widespread dissemination of *M tuberculosis* occurs) and opportunistic infections in HIV co-infection could complicate critical illness further. Very young children (younger than age 1 year) are highly susceptible to *M tuberculosis*, and might present acutely and deteriorate rapidly.⁷ Hydrocephalus is particularly common in children, affecting 931 of 1088 children with tuberculous meningitis in a systematic review of nine studies.⁹

Monitoring of critically ill patients with tuberculous meningitis

The continuous monitoring of physiological variables (temperature, respiratory rate, pulse, and blood pressure) should be the standard of care in critical illness, although this is probably hard to achieve in resource-limited settings. In this Review, we focus on additional monitoring specific to tuberculous meningitis, and discuss the most basic (and most available) non-invasive monitoring methods first, followed by the invasive techniques (table 2).

Clinical assessment and basic bedside monitoring

Clinical assessment and basic bedside monitoring can sometimes be the only available monitoring techniques in some resource-limited settings. The Glasgow Coma Scale is easy to learn, reliable with training, and recognised internationally.⁵⁵ In children younger than age 5 years, in whom verbal and motor abilities are less developed, a modified Glasgow Coma Scale might be more suitable. The use of the Glasgow Coma Scale to monitor treatment response might be confounded by intubation, sedative drugs, and pre-existing neurological conditions (such as dementia or psychiatric disorders).⁵⁵ The UK Medical Research Council tuberculous meningitis grade combines the Glasgow Coma Scale and focal neurological signs to categorise disease severity.⁷ A high tuberculous meningitis grade at presentation predicts mortality regardless of HIV status.³⁷ EEG monitoring is recommended in critically ill individuals with encephalitis who are comatose,⁶⁰ although the role of this tool in monitoring of critically ill patients with tuberculous meningitis is unclear.

Non-invasive intracranial pressure monitoring

Transcranial Doppler ultrasound uses a low frequency transducer, placed on the scalp, to determine cerebral blood flow velocity in the basal arteries of the brain, with

For more on GeneXpert see https://www.cdc.gov/tb/publications/factsheets/pdf/xpertmtb-rifassayfactsheet_final.pdf

	Therapeutic role in tuberculous meningitis	Primary adverse effect	Drug interactions	Additional adverse effects
Rifampicin	First-line antituberculosis therapy	Induces cytochrome 3A4 enzyme ⁴²	Antiretroviral therapy drugs (including non-nucleoside reverse transcriptase inhibitors and protease inhibitors), reduces serum concentrations; benzodiazepines, substrates for cytochrome 3A4	Drug-induced liver injury; hypersensitivity including Stevens-Johnson syndrome; renal failure; adrenal insufficiency; haemolysis; cytopenia
Isoniazid	First-line antituberculosis therapy	Inhibits cytochrome 3A4 enzyme ⁴²	Phenytoin, higher serum phenytoin concentrations in slow acetylators; ⁴³ benzodiazepines, substrates for cytochrome 3A4	Drug-induced liver injury; seizures; psychiatric disorders; toxic epidermal necrolysis; Stevens-Johnson syndrome; pancreatitis; haemolysis; cytopenia
Pyrazinamide	First-line antituberculosis therapy	Drug induced liver injury ⁴¹	No interactions relevant to critically ill patients with tuberculous meningitis	Hypersensitivity, including urticaria
Ethambutol hydrochloride	First-line antituberculosis therapy	Ocular toxicity difficult to detect in comatose patients ⁷	No interactions relevant to critically ill patients with tuberculous meningitis	Hypersensitivity, including Stevens-Johnson syndrome; thrombocytopenia; leucopenia; renal failure
Fluoroquinolones (eg, levofloxacin, moxifloxacin hydrochloride, gatifloxacin)	Second-line antituberculosis therapy	Lowers seizure threshold ⁴⁵	No interactions relevant to critically ill patients with tuberculous meningitis	Drug-induced liver injury; seizures; psychiatric disorders; QT prolongation; toxic epidermal necrolysis; Stevens-Johnson syndrome; haemolysis; cytopenia; renal failure
Corticosteroids (eg, dexamethasone)	Adjunctive antiinflammatory drug	Associated with an increased risk of gastrointestinal bleeding in patients admitted to hospital ⁴⁴	Aspirin, adverse effect of corticosteroids overlaps with treatment effect of aspirin	Adrenal insufficiency on discontinuation; psychosis; infections
Aspirin	Anti-platelet and antithrombotic agent	Increased gastrointestinal bleeding tendencies established	Corticosteroids, treatment effect of aspirin overlaps with adverse effect of corticosteroids	Hypersensitivity
Acetazolamide	Reduces CSF production	Might cause hyponatraemia, or exacerbate hyponatraemia of tuberculous meningitis ⁴⁶	Overlapping treatment effect profile with fludocortisone; increased metabolic acidosis and neurological effects with concomitant aspirin; interactions with anti-convulsants	Electrolyte imbalance; renal failure; extensive adverse effects and drug interactions

Table 1: Drugs commonly used during management of tuberculous meningitis

intracranial pressure possibly inferred from changes in the pulsatility index.⁵² Transcranial Doppler ultrasound has been used to detect vasculopathy in patients with tuberculous meningitis,⁵³ although its value in quantifying intracranial pressure is uncertain given that changes in partial pressure of carbon dioxide or blood pressure could alter blood flow and pulsatility index, independently of changes in intracranial pressure.⁵⁷ A study of 20 children from South Africa showed that pulsatility index could not reliably predict intracranial pressure in children with tuberculous meningitis.⁶²

Optic nerve sheath diameter ultrasound is a potentially quick, easy, and safe method for early intracranial pressure detection. The optic nerve is surrounded by a dural sheath that distends in its retrobulbar segment under elevated intracranial pressure. Optic nerve sheath appearances can be recorded and distension measured (figure 1). In patients with tuberculous meningitis, higher optic nerve sheath diameter measurements have been associated with raised intracranial pressure.⁵⁸ No data yet exist to support the use of optic nerve sheath diameter measurement in

guiding management, measuring treatment response, or improving outcomes in patients with tuberculous meningitis.

Lumbar puncture

Lumbar puncture and CSF analysis are essential for diagnosis of tuberculous meningitis and can be used to assess treatment response, provide opening pressure measurement, and enabling air encephalography. CSF opening pressure is elevated in approximately half of adult tuberculous meningitis cases,⁶³ although no evidence yet supports opening pressure as a predictor of intracranial pressure or outcome in patients with tuberculous meningitis. Low pretreatment leucocyte numbers and CSF glucose concentrations have been associated with death from tuberculous meningitis,⁵ and CSF glucose concentrations rise with successful treatment. However, the value of repeated assessments of CSF opening pressure, glucose concentrations, and leucocyte numbers in defining clinical management and prognosis has not yet been studied.

	Advantages	Disadvantages
Non-invasive		
Glasgow Coma Scale	Well known internationally; generally reproducible; easy to learn; and effective for regular assessment ⁴⁵	Broad tool for assessment; unlikely to identify subtle changes in clinical status; influenced by reversible (drugs such as sedatives) and irreversible factors (cranial nerve palsies) so sensitivity will change with time; cannot be applied to children younger than age 5 years ⁴⁵
CT brain imaging	Identifies hydrocephalus and large mass lesions; available at most large centres; fast procedure	Role in identifying raised intracranial pressure uncertain; might not identify subtle changes in intracranial pressure; often requires contrast; radiation exposure
MRI brain imaging	Likely to identify intracranial cause of neurological deterioration in patients with tuberculous meningitis; good sensitivity for detecting pathological features of tuberculous meningitis; can detect acute infarcts with diffusion weighted imaging; multiple imaging sequences available allowing targeted detection of specific pathologies	Role in identifying raised intracranial pressure uncertain; time consuming; might be unavailable, or MRI machine could be located off site with patient transfer to machine time consuming and high risk of patient deterioration during transit; might require anaesthesia in young patients
Transcranial Doppler ultrasound	Safe procedure	Value in quantifying intracranial pressure is uncertain, but useful to detect vasculopathy in patients with tuberculous meningitis; ⁴⁶ only measures cerebral blood flow in major vessels in Circle of Willis, which might change (via changes in partial pressure of carbon dioxide or mean arterial pressure) independently of intracranial pressure; ⁴⁷ user dependent
Optic nerve sheath diameter ultrasound	Ultrasound machines available at many centres; fast and safe procedure	Requires knowledge of normal population values for comparison; not for continuous monitoring; role yet to be defined in management, but a study of 25 patients with tuberculous meningitis showed promising results ⁴⁸
Invasive		
Lumbar puncture	Single pressure measurement (opening pressure) well recognised; equipment widely available; allows CSF drainage; allows air encephalogram to be performed	Association with intracranial pressure changes uncertain; continuous pressure measurement available only at specialist centres
Intraventricular catheters, intraparenchymal pressure transducers, subarachnoid bolts, and epidural transducers	Gold standard for intracranial pressure monitoring; continuous monitoring allows rapid detection of intracranial pressure changes; ventricular catheters allow CSF drainage	Risks of infection and bleeding; ⁴⁹ available only at specialist centres; costly

Table 2: Methods of intracranial pressure monitoring in patients with tuberculous meningitis

Invasive intracranial monitoring devices

Gold standard intracranial pressure monitoring is invasive and can be done at intraventricular, intraparenchymal, and epidural sites.⁵⁹ Haemorrhage and infection are rare risks of intracranial device insertion, and devices should not be inserted in individuals with coagulopathy or at sites of local infection.⁶⁴ Intraventricular access allows CSF drainage, sampling, and drug administration.⁵⁹ Descriptions of intracranial pressure monitoring in patients with tuberculous meningitis are scarce, and whether intracranial pressure monitoring guides treatment regimen or improves outcomes is uncertain.

Biomarkers of brain injury

Biomarkers of neuronal injury offer a potential future approach to quantifying and monitoring brain pathology. Brain pathologies result in leakage of proteins into the CSF where they can be measured, or into the bloodstream through an impaired blood–brain barrier.⁶⁵ A study of blood–brain barrier function and CSF biomarkers of CNS injury in 66 adults and children with brain infections in Laos found blood–brain barrier leakage and higher amounts of glial fibrillary acidic protein in the 11 adults with tuberculous meningitis than was observed in the

patients with other brain infections (except bacterial meningitis).⁶⁶ An increasing concentration of CSF biomarkers of neuronal and glial injury over time was associated with worse outcome in a study of 44 paediatric patients with tuberculous meningitis and hydrocephalus in South Africa.⁶⁶ Biomarkers represent a potential future method to monitor treatment response and predict outcome; however, further research is required to define optimal concentration ranges of biomarkers for clinical use.

Brain imaging

Brain imaging allows identification of the causes (hydrocephalus, tuberculous masses, cerebral oedema) and consequences (brain shift and ischaemia) of raised intracranial pressure. Baseline brain imaging can alter immediate management if hydrocephalus is found, and follow-up brain imaging is recommended for patients with worsening symptoms.⁷ No evidence supports routine follow-up brain imaging in patients who recover back to neurological baseline from tuberculous meningitis. Brain CT can identify dilated ventricles, mass effects, infarctions, and inflammatory exudates. The detrimental effects of brain irradiation from CT during brain injury remain

unknown. Brain MRI has no radiation risks and provides higher resolution of *M tuberculosis*-associated brain pathology than does CT, although it is expensive, time

consuming, and might require transfer of a critically ill patient to a hospital with MRI.^{68,69} By contrast, enhanced fluid attenuated inversion recovery MRI, removal of CSF enhancement (and removal of enhancement of normal vessels and meninges) might show inflammation and leptomeningeal changes more clearly than does CT.^{70,71} Retrospective data show that both CT and MRI have a role in detecting brain pathology associated with tuberculous meningitis.⁷² In a study of 26 adults and children with tuberculous meningitis, magnetic resonance angiography identified blood vessel abnormalities in 11 (42%) of 26 patients,⁷³ suggesting a future role of this imaging technique in monitoring of tuberculous meningitis.

Management of critically ill patients with tuberculous meningitis

This section on management of critical illness starts with supportive management, followed by medical, and then surgical management (panel 2).

General patient management

Supportive care is critical in tuberculous meningitis, yet few data exist to guide management. With control of intracranial pressure a priority, supportive care aims to optimise patient position and parameters such as temperature, haemoglobin, and glucose concentrations, and avoid complications of prolonged critical illness such as ventilator associated pneumonia and pressure area damage (panel 3). Fever is associated with worse outcomes in patients with severe neurological injury of multiple causes,⁷⁴ and an increased 1-year mortality in HIV-negative individuals with tuberculous meningitis.⁵ Cerebral metabolic rate and oxygen demand fall with body temperature,⁷⁵ and a role for therapeutic hypothermia in neuroprotection has been explored. RCTs showed that therapeutic hypothermia was associated with increased mortality in patients with severe bacterial meningitis,⁷⁶ and worse functional outcomes in patients with traumatic brain injury;⁷⁸ however, no trials have investigated therapeutic hypothermia in patients with tuberculous meningitis.

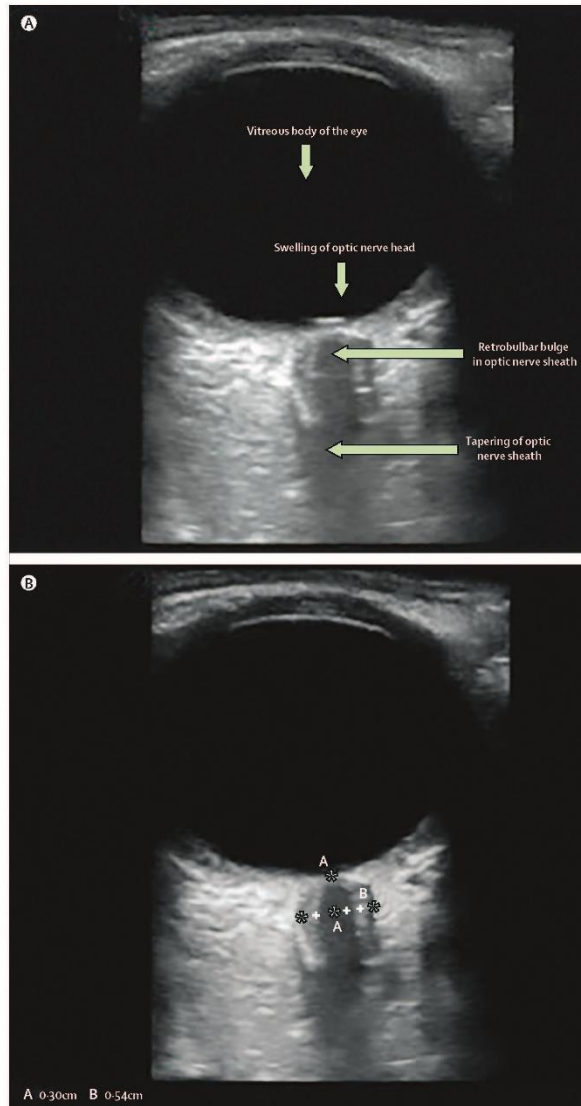


Figure 1: Use of optic nerve sheath diameter ultrasound in critically ill patients with tuberculous meningitis

Optic nerve sheath diameter ultrasound is a simple, non-invasive tool that shows potential to screen individuals with tuberculous meningitis for raised intracranial pressure.¹⁹ (A) Optic nerve sheath diameter ultrasound image of the left eye from an adult patient with Medical Research Council grade 2 tuberculous meningitis (GCS 11–14, or GCS 15 with focal neurological signs) and raised intracranial pressure. This image was taken at diagnosis of tuberculous meningitis, on the first day of antituberculosis chemotherapy. At the posterior aspect of the globe, a short narrow horizontal line represents swelling of the head of the optic nerve. Retrobulbar distension of the optic nerve sheath can be seen, with a subsequent tapering of the optic nerve sheath proximally. (B) Identical to (A) with the addition of measuring calipers, showing retrobulbar distension of the optic nerve sheath measured at 0.3 cm from the posterior aspect of the globe of the eye (A–A calipers), and the diameter of the optic nerve sheath (caliper B, diameter 0.54 cm).

Raised intracranial pressure contributes to poor outcome in patients with tuberculous meningitis. Intracranial pressure reduction strategies derive from evidence acquired in other causes of brain injury. We summarise potential strategies for optimising intracranial pressure and preserving brain perfusion in critically ill individuals with tuberculous meningitis (figure 2). Supportive therapies include optimisation of patient position for CSF and cerebral venous drainage, avoidance of hyperthermia and hypotension, control of seizures, and appropriate mechanical ventilation. Drug therapies include acetazolamide for reducing CSF production, adjunctive anti-inflammatory therapy, and antituberculosis chemotherapy. Furthermore, sodium management, hyperosmolar therapy, and neurosurgical strategies (endoscopic third ventriculostomy [ETV] and ventriculoperitoneal [VP] shunting) are also potential treatment strategies.

Airway protection and respiratory failure

In two studies, 53 (70%) of 76 adults and children¹⁶ and 63 (70%) of 90 adults¹⁹ with tuberculous meningitis, admitted to an intensive care unit, required mechanical ventilation. However, optimal ventilation strategies in patients with tuberculous meningitis are unknown. Neuromuscular blockade could allow more efficient control of oxygenation, hyperventilation, and positive end-expiratory pressure, and limit coughing, with the overall result of avoiding surges of intracranial pressure; but no data support its routine use in patients with traumatic brain injury²⁰ or those with tuberculous meningitis. Hypocarbica and hypercarbica have detrimental effects on cerebral blood flow and intracranial pressure in patients with tuberculous meningitis.⁷ No evidence supports the use of hyperventilation for intracranial pressure management in patients with tuberculous meningitis.

Antituberculosis chemotherapy

The use of antituberculosis chemotherapy in patients with tuberculous meningitis has been reviewed elsewhere⁷ and only aspects relevant to critically ill patients with tuberculous meningitis are discussed in this Review. Rifampicin, isoniazid, pyrazinamide, and ethambutol are first-line agents for drug-susceptible tuberculosis,⁷ with 9–12 month regimens common for patients with tuberculous meningitis.² Prompt treatment and avoidance of therapy interruptions are essential to reduce mortality from tuberculous meningitis.⁷ Optimal drug doses and administration routes are unknown, especially in critically ill patients, although some evidence has shown that high rifampicin doses (>10 mg/kg, normal dose is 10 mg/kg per day, up to a maximum of 600 mg per day) administered intravenously might improve outcomes.¹⁴ Severely ill individuals who are unable to swallow can receive crushed antituberculosis medications via an enteral feeding tube, yet no data exist confirming that this approach achieves adequate intracerebral drug concentrations. Intravenous preparations might be preferable, if available, although

Panel 2: Case study

A 30-year-old man was admitted to a hospital in Ho Chi Minh City, Vietnam, with a 2-week history of fever, headache, and meningism. He had no relevant past medical history. Glasgow Coma Scale score was 15, and the only abnormal neurological sign was a right sixth cranial nerve palsy. Serum sodium was 117 mmol/L and liver function tests were normal. An HIV test was positive, CD4 count was 17 cells per μ L, and a chest x-ray was normal. CSF analysis revealed 304 white blood cells (73% neutrophils), protein was 2.01 g/L, glucose was 0.72 mmol/L (paired serum glucose was 4.75 mmol/L), and lactate was 7.7 mmol/L. Acid-fast bacilli were seen on CSF smear microscopy and a CSF GeneXpert test was negative. CT (appendix) and MRI brain imaging with contrast were normal. Tuberculous meningitis was diagnosed and first-line oral antituberculosis chemotherapy was initiated (rifampicin, isoniazid, pyrazinamide, and ethambutol, at weight appropriate doses). Adjunctive dexamethasone was used, initially at 0.4 mg/kg per day, with weekly tapering. Reduced serum osmolality, inappropriately high urine osmolality, elevated urinary sodium, and hypovolaemia were consistent with cerebral salt wasting, and hypertonic saline was administered to correct circulating volume and serum sodium concentration. Antiretroviral therapy was not immediately started because of the risk of neurological immune reconstitution inflammatory syndrome.

2 weeks after starting antituberculosis chemotherapy, the patient's condition had improved. The cranial nerve palsy had resolved, serum sodium had recovered to 130 mmol/L with correction of hypovolaemia, and hypertonic saline therapy had been stopped. A decision was made to commence antiretroviral therapy (tenofovir, lamivudine, and efavirenz). After 5 days, the patient developed a headache and began vomiting. Glasgow Coma Scale score remained at 15. Brain CT with contrast did not reveal focal lesions, although cerebral ventricles had slightly increased in size (appendix). After 5 days, the Glasgow Coma Scale score acutely fell to 11 (eyes open to speech, inappropriate words, localising motor response). MRI brain imaging with contrast showed substantially enlarged cerebral ventricles (appendix) with a tuberculoma compressing the fourth ventricle outlet. Antituberculosis chemotherapy was administered via a nasogastric tube, and high dose intravenous dexamethasone was initiated. A decision was made in conjunction with the patient's family and neurosurgical team not to proceed with neurosurgical intervention. The following day, the Glasgow Coma Score fell to 7 and the patient underwent endotracheal intubation and mechanical ventilation. Supportive care and medical therapy were continued. 2 days later, the patient had a cardiac arrest and died.

evidence is scarce, and no intravenous pyrazinamide preparation exists. Interruptions to first-line antituberculosis chemotherapy could be necessary if drug-induced liver injury occurs, although the transaminase thresholds for stopping drugs, and the timing of their reintroduction, have not yet been defined by RCTs.

Anti-inflammatory treatment

Adjunctive corticosteroids, given from the start of antituberculosis drug treatment, reduce mortality from tuberculous meningitis, at least in the short term (less than 2 years after treatment initiated).³⁰ Their benefit in HIV co-infection is uncertain and is the objective of an ongoing RCT in Vietnam and Indonesia (NCT03092817).³¹ The optimal dose and administration route, and whether prednisolone and dexamethasone are equally effective, remain unknown. Correct use of corticosteroids following the start of antituberculosis drug treatment in the management of complications causing neurological

Panel 3: Approaches and evidence gaps in the supportive care of critically ill patients with tuberculous meningitis**Infection control and reducing potential in-hospital***Mycobacterium tuberculosis* transmission

- Respiratory isolation might be required in patients with concomitant pulmonary tuberculosis
- Respiratory isolation in critical care is challenging when patients require constant monitoring from staff
- The effect of endotracheal intubation and a closed ventilation circuit on tuberculosis transmission is uncertain, given that periodic breaks to this closed circuit to allow suction might induce coughing

Head-of-bed elevation to reduce intracranial pressure

- Elevating the head end of a bed is known to aid venous drainage and shifts CSF extracranially, but might also lower mean arterial pressure
- Optimal head-of-bed elevation in patients with tuberculous meningitis has not been studied

Maintaining normal glucose concentrations

- Hyperglycaemia and hypoglycaemia adversely affect the brain during critical illness; however, optimal glucose control is not known²⁴

Treating anaemia to ensure optimal tissue oxygenation

- Haemoglobin is important for optimal oxygen delivery in patients at risk of ischaemia, and optimal transfusion thresholds have not been determined in patients with tuberculous meningitis

Controlling and reducing fever

- Whether treating fever in patients with tuberculous meningitis improves outcomes is not known
- No randomised controlled trials have yet described therapeutic hypothermia in patients with tuberculous meningitis

Preventing deep vein thrombosis

- The role of deep vein thrombosis prophylaxis in critically ill patients with tuberculous meningitis, in which corticosteroids and aspirin might add to the gastrointestinal bleeding risk, has not been studied
- The effect of head-of-bed elevation or general patient positioning in patients with tuberculous meningitis on deep vein thrombosis has not been studied

Protecting pressure areas

- The effect of head-of-bed elevation or general patient positioning on pressure area damage in patients with tuberculous meningitis has not been studied

Ventilator-associated pneumonia prevention

- Mechanical ventilation is common in critical care tuberculous meningitis²⁹
- Almost a quarter of patients with tuberculous meningitis who had mechanical ventilation developed ventilator-associated pneumonia²⁹
- Ventilator-associated pneumonia prevention strategies are uncertain in patients with tuberculous meningitis

Optimising nutrition

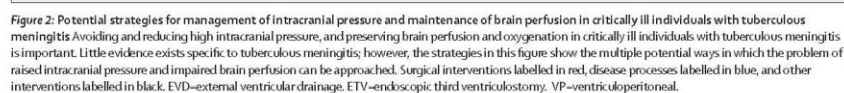
- Tuberculosis is a catabolic illness, yet no nutritional guideline exists specifically for tuberculous meningitis
- Alternative drug administration routes can be considered when an oral route is unavailable in unconscious patients

deterioration and critical illness is unclear. Despite weak evidence, corticosteroids are often used as rescue therapy for raised intracranial pressure as they can reduce cerebral oedema. They are often used in the treatment of neurological deterioration caused by expanding brain tuberculomas³⁵ and for immune reconstitution inflammatory syndrome in patients with HIV.⁵⁰ Occasionally, tuberculomas do not respond to corticosteroids, with persistence or progression of symptoms associated with tuberculomas despite therapy.⁷ Case reports and small case series suggest adjunctive thalidomide, infliximab, and interferon gamma might be effective in this circumstance.⁸ Adjunctive aspirin given with dexamethasone might reduce brain infarcts and improve mortality, and phase 3 trials are planned to assess the effect of adjunctive aspirin administered with dexamethasone on brain infarcts and mortality.²⁹

Seizure management

Data regarding the cause and timing of seizures in patients with tuberculous meningitis is scarce, and their

incidence appears to vary substantially between populations. A study of 817 Vietnamese adults with tuberculous meningitis showed that seizures occurred in 11 (3%) of 409 patients receiving standard antituberculosis chemotherapy.¹⁵ Seizures were also reported in 8% of 515 HIV-negative individuals and 13% of 93 HIV-positive individuals (all older than age 14 years) with tuberculous meningitis in Indonesia.⁶ Conversely, a study of Indian adults with tuberculous meningitis showed seizures in 27 (34%) of 79 patients, and abnormal EEG changes were observed in 17 (85%) of 20 patients who had seizures and EEG tests.⁷⁸ Early-onset seizures were associated with cerebral oedema and meningeal irritation, whereas late-onset seizures were associated with hydrocephalus, cerebral infarction, and tuberculomas in patients with tuberculous meningitis.⁷⁸ Seizure risk can be increased by fluoroquinolone coadministration.^{15,46} The optimum treatment of seizures in patients with tuberculous meningitis has not been studied. Therapy is complicated when cytochrome P450 induction and inhibition alters concentrations of drugs metabolised by these



restriction in a critically ill patient with cerebral salt wasting is potentially harmful because these patients are often profoundly hypovolaemic. Assessment of intravascular fluid status can guide therapy, with hypovolaemia expected in patients with cerebral salt wasting, and euvolaemia expected in patients with syndrome of inappropriate antidiuretic hormone secretion.³⁴

Maproprate and indinavir normalise secretion. Meta-analyses of studies in patients with traumatic brain injury suggest mannitol could have a detrimental effect on mortality when compared with hypertonic saline (four studies),⁸⁴ or no benefit of one intervention over the other (six studies).⁸⁵ Hypertonic saline might reduce intracranial pressure faster, to a greater extent, and for longer than does mannitol; however, the choice of agent requires individual patient consideration.⁸⁶ Case reports describe use of mannitol and hypertonic saline in patients with tuberculous meningitis,^{87,88} but no comparative clinical trials exist. Mannitol can promote hypovolaemia (reducing cerebral perfusion pressure) through dehydration, and the osmotic gradient reversal upon stopping mannitol is potentially harmful. Osmotic properties of hypertonic saline mean it is less likely to cross the blood-brain barrier than is mannitol,⁸⁶ which reduces the ability of hypertonic saline to exacerbate raised intracranial pressure. Rapid correction of hyponatraemia could result in central pontine myelinolysis, which reduces the ability of and would result in worsening of clinical neurological state.⁸⁴

Hyponatraemia and hyposmolal therapy
Hyponatraemia commonly accompanies critical illness resulting from tuberculous meningitis, and cerebral salt wasting and syndrome of inappropriate antidiuretic hormone secretion are considered the most likely causes.³⁵ An RCT compared intravenous and oral sodium supplementation with or without fludrocortisone (0.1–0.4 mg/day) in the treatment of 37 Indian adults with hyponatraemia (<135 mEq/L) caused by cerebral salt wasting associated with tuberculous meningitis.³³ Fludrocortisone (combined with intravenous and oral salt supplementation) was significantly associated with faster correction of plasma sodium than intravenous and oral salt supplementation alone (4 days vs 15 days), but did not influence mortality or disability at 6 months. Fludrocortisone was associated with severe hypokalaemia and hypertension in two patients with hyponatraemia, necessitating its discontinuation.³³ For syndrome of inappropriate antidiuretic hormone secretion, clinical practice guidelines recommend fluid restriction as first-line treatment,³⁴ although this approach has not been investigated in patients with tuberculous meningitis. Distinguishing the cause of hyponatraemia in patients with tuberculous meningitis is difficult,³⁵ and fluid

Hydrocephalus

Differentiation of communicating from non-communicating hydrocephalus is important, yet difficult with conventional brain imaging techniques such as CT and MRI. Air encephalography shows whether intrathecally injected air can pass into the lateral ventricles of the brain,⁷ yet is rarely done. Acetazolamide reduces CSF production,⁴⁶ and could have value in hydrocephalus treatment, but no recent trials of medical treatment of communicating hydrocephalus, with acetazolamide or other therapy, have been done. Thus, optimal drug therapy for communicating hydrocephalus is unknown.

Urgent neurosurgical intervention relieves high intracranial pressure in non-communicating hydrocephalus, and could be tried for communicating hydrocephalus in cases for which medical therapy is ineffective. The most commonly used surgical procedures are external ventricular drainage (EVD), ETV, and VP shunting, described in detail elsewhere.⁴⁹ EVD can temporarily relieve acutely raised CSF pressure in patients who might not require long-term hydrocephalus treatment. ETV is an endoscopic procedure that connects obstructed CSF in the ventricular system to the pre-pontine cistern through a stoma, allowing access to possibly normal CSF absorption areas of the brain.⁵⁰ ETV is technically more difficult in patients with acute tuberculous meningitis than in other causes of hydrocephalus because of the increased exudate in the basal cisterns, and outcomes can improve when the procedure is done at a later stage during disease progression.⁵⁰

ETV and VP shunting carry a substantial risk of bleeding and infection,⁵¹ and are only available in specialist centres. An RCT comparing ETV versus VP shunting in 48 children (≤ 18 years) with hydrocephalus associated with tuberculous meningitis in India showed that ETV was successful in ten (42%) of 24 patients, and VP shunting successful in 13 (54%) of 24 patients.⁵⁰ Although no significant difference in success was observed between these two surgical techniques, the timing of the procedure (days of anti-tuberculosis chemotherapy received) must be considered along with procedural success and risks of the procedure after the relevant number of days of antituberculosis treatment. ETV and VP shunting were compared in an observational study of 52 children (<18 years)⁵² with hydrocephalus associated with tuberculous meningitis in India. ETV was successful in 17 (65%) of 26 patients and VP shunting successful in 16 (62%) of 26 patients. Unsuccessful cases of both techniques were linked to disease severity, emphasising the challenges of surgically managing severe cases of tuberculous meningitis.⁵² An age of younger than 5 years was significantly associated with VP shunt failure.⁵² A systematic review of 19 studies including 1038 patients with hydrocephalus associated with tuberculous meningitis concluded that high-quality data in adults for these techniques are scarce.⁵³ Long-term outcomes in hydrocephalus associated with tuberculous meningitis are

worse in individuals with HIV,⁵³ although better outcomes are suggested in those receiving antiretroviral therapy.⁵⁴ Decision making between medical treatment of hydrocephalus, EVD, ETV, and VP shunting remains challenging and controversial.

Conclusions and future research

The management of critically ill patients with tuberculous meningitis is difficult. Conventional triggers for intensive care unit admission (such as reduced Glasgow Coma Scale score) might occur too late in the disease process, and for tuberculous meningitis prognosis quickly worsens once coma develops. Coma is often the result of raised intracranial pressure due to hydrocephalus, infarction, or tuberculoma mass effect.⁷ A management priority is to monitor and safely reduce intracranial pressure, preserving brain perfusion while antituberculosis chemotherapy takes effect. The high mortality and morbidity associated with tuberculous meningitis might result from the disease itself, its complications, or its treatment, all of which can contribute to critical illness. Complications (eg, tuberculomas) could be present at diagnosis,^{5,55} or develop after beginning antituberculosis chemotherapy.⁵¹ RCTs are required to provide an evidence base for the management of critically ill patients with tuberculous meningitis. The benefit of therapies that are effective in other conditions such as hypertonic saline²² remain to be proven in patients with tuberculous meningitis. The development of a staircase approach to manage intracranial pressure in patients with tuberculous meningitis, similar to that proposed for those with traumatic brain injury,²² would be valuable.

Hydrocephalus, hyponatraemia, and supportive management are crucial areas to target for future research. The distinction between communicating and non-communicating hydrocephalus is essential to guide therapy. Future research should investigate the use of air encephalograms to make this distinction, whether this practice is safe, affects therapy, and improves outcomes are other aspects of further investigation. Paediatric studies^{50,51} have reported varying success with ETV and VP shunting for treatment of hydrocephalus. RCTs of acetazolamide with or without frusemide in the treatment of communicating hydrocephalus are required. An improved understanding of hyponatraemia's pathophysiology is important to guide care of patients with tuberculous meningitis. The effects on patient outcome of treating hyponatraemia, or further sodium reduction after correction are not known. No evidence supports fluid restriction for syndrome of inappropriate antidiuretic hormone secretion associated with tuberculous meningitis. For individuals receiving hypertonic saline, the optimum saline concentration, total daily dose and volume, and duration of therapy are unknown. Clinical parameters to guide therapy—extracellular or intravascular volume, serum sodium, or other parameters—are uncertain. These could be assessed in future clinical

trials, in which hypertonic saline is administered to volume or sodium targets, and serum sodium, intracranial pressure, and neurological outcome are endpoints. The potential benefit and harm of fludrocortisone was shown in a small study³³ and a larger trial is needed.

Evidence for supportive care specific to patients with tuberculous meningitis is weak. Optimal infection control strategies, nutrition, and pressure area care are unknown, and further study is warranted. Head-of-bed elevation angles should be studied as this practice reduces intracranial pressure and less gastric secretions move into lungs, which can cause ventilator-associated pneumonia. Study endpoints could include pressure area damage (sacral skin and tissue that ulcerates when patients are bedbound for long periods), ventilator-associated pneumonia, and neurological outcomes (neurological disability and mortality). Furthermore, the effect of seizure prophylaxis and thromboprophylaxis upon drug interactions, seizures, venous thromboembolism, and gastrointestinal bleeding needs to be assessed in prospective studies. A supportive tuberculous meningitis care bundle containing supportive strategies to follow and therapies to administer could be created and studied. Ward round checklists specific to patients with tuberculous meningitis could ensure daily review and improvements in all aspects of care.

Anti-inflammatory therapy is widely used in patients with tuberculous meningitis, yet the optimal dose and duration is not known for adults or children.^{23,8} RCTs of corticosteroid therapy are required for both paradoxical reactions and IRIS to provide strong evidence to support their use in these conditions, and to guide dosing and treatment duration. The optimal dose and duration of corticosteroids in patients with tuberculous meningitis is not known for adults or children. Additionally, the effect of tapering therapy on adrenal function remains unknown.

Mortality prediction in patients with tuberculous meningitis is improving,^{3,37} and could identify high-risk individuals requiring intensive monitoring. For example, in a retrospective study of brain infection in a critical care unit, in which 36 (47%) of 76 patients had tuberculous meningitis, duration of hospital stay or mechanical ventilation predicted mortality.¹⁶ Early identification of complications associated with tuberculous meningitis through intensive monitoring can allow earlier therapy and improve outcomes. Non-invasive intracranial pressure monitoring has shown promise as a tool for the identification of raised intracranial pressure in patients with tuberculous meningitis;³⁸ however, robust evidence that techniques such as optic nerve sheath diameter ultrasound detect raised intracranial pressure earlier, lead to appropriate interventions, and then improve outcomes, is required. A future trial design could compare standard care with care in which optic nerve sheath diameter ultrasound is used. A diameter value over a set threshold would lead to brain imaging followed by appropriate

Search strategy and selection criteria

We searched PubMed for human studies published in any language between Jan 1, 2013, and Jan 14, 2019, using the search terms: "neurocritical care" (variations: "neuro critical care", "neurointensive care", "neuro intensive care", "critical care", "intensive care") or "tuberculous meningitis" (variations: "tuberculosis meningitis", "TB meningitis", "TBM"), plus each one of the following search terms: "admission", "clinical trials", "monitoring", "transcranial Doppler ultrasound", "TCD ultrasound", "optic nerve sheath diameter ultrasound", "ONSD ultrasound", "head of bed elevation", "nutrition", "infection control", "anti-tuberculosis chemotherapy", "antitubercular chemotherapy", "antiretroviral therapy", "ART", "corticosteroids", "anti-inflammatory", "aspirin", "sedatives", "anticonvulsants", "hyperthermia", "hypothermia", "seizure", "mechanical ventilation", "tracheostomy", "hyponatraemia", "hyperosmolar therapy", "hypertonic saline", "mannitol", "fludrocortisone", "acetazolamide", "hydrocephalus", "cerebral infarction", "stroke", "cerebrovascular accident", "CVA", "cerebral ischaemia", "tuberculoma", "immune reconstitution inflammatory syndrome", "IRIS", "paradoxical reaction", "HIV co-infection", "brain imaging", "computed tomography", "CT", "magnetic resonance imaging", "MRI", "electroencephalogram", "EEG", "neuromonitoring", "positron emission tomography imaging", "PET imaging", "18F-FDG PET", "jugular venous saturation monitoring", "brain tissue oxygen tension monitoring", "PbtO2", "near infra-red spectroscopy", "biomarkers of brain injury", "intracerebral microdialysis", "transcranial cerebral oximetry", "lumbar puncture", "opening pressure", "severe", "critical", "coma", or "standardisation". A separate search of PubMed was done using the search terms "neurocritical care" (variations: "neuro critical care", "neurointensive care", "neuro intensive care", "critical care", "intensive care") and "tuberculous meningitis" (variations: "tuberculosis meningitis", "TB meningitis", "TBM") only. Relevant references of selected papers were also included. The final list of references was based on relevance to the topics covered in this Review.

therapy on the basis of imaging findings, with neurological disability and mortality recorded as outcomes. Preliminary research monitoring (such as 18F-fluorodeoxyglucose PET, jugular venous saturation monitoring, and interstitial fluid sampling; appendix) show promise for the future, and further data regarding their role in monitoring of patients with tuberculous meningitis should be collected.

Contributors

GET and JD defined the concept and scope of the Review, with discussion with AF and UR. JD did the literature review and wrote the first draft of the manuscript. JD, AF, DI, NHP, UR, and GET reviewed and revised the manuscript drafts and agreed on the final manuscript for submission.

Declaration of interests

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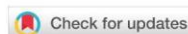
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See Online for appendix

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OPEN LETTER

Checklists to guide the supportive and critical care of tuberculous meningitis [version 2; peer review: 2 approved]

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Abstract

The assessment and management of tuberculous meningitis (TBM) is often complex, yet no standardised approach exists, and evidence for the clinical care of patients, including those with critical illness, is limited. The roles of proformas and checklists are increasing in medicine; proformas provide a framework for a thorough approach to patient care, whereas checklists offer a priority-based approach that may be applied to deteriorating patients in time-critical situations.

We aimed to develop a comprehensive assessment proforma and an accompanying 'priorities' checklist for patients with TBM, with the overriding goal being to improve patient outcomes. The proforma outlines what should be asked, checked, or tested at initial evaluation and daily inpatient review to assist supportive clinical care for patients, with an adapted list for patients in critical care. It is accompanied by a supporting document describing why these points are relevant to TBM. Our priorities checklist offers a useful and easy reminder of important issues to review during a time-critical period of acute patient deterioration. The benefit of these documents to patient outcomes would require investigation; however, we hope they will promote standardisation of patient assessment and care, particularly of critically unwell individuals, in whom morbidity and mortality remains unacceptably high.

Keywords

Tuberculous meningitis, critical care, checklist, proforma

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This article is included in the [Tuberculous Meningitis International Research Consortium](#) collection.

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REVISED Amendments from Version 1

Under respiratory monitoring we have added here that pneumothorax is rare in PJP (with a reference).

In Table 4 we have added 'preferably with contrast' in reference to brain imaging.

In Table 4 we have specifically mentioned IRIS and cerebral sinus thrombosis. In the supporting document we have added the sentence 'Cerebral venous thrombosis is an unusual cause of acute neurological deterioration in TBM, but has been described' with references.

Regarding risks of prolonged hospital admission, we have added 'Deep vein thrombosis is a risk of prolonged critical care admission'.

Any further responses from the reviewers can be found at the end of the article

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Abbreviations

AFB, acid-fast bacilli; ALT, alanine transaminase; ART, anti-retroviral therapy; AST, aspartate aminotransferase; BCG, Bacillus Calmette Guérin; CO₂, carbon dioxide; CBF, cerebral blood flow; CPP, cerebral perfusion pressure; CRP, C-reactive protein; CSF, cerebrospinal fluid; CSW, cerebral salt wasting; CT, computed tomography; CVP, central venous pressure; ESR, erythrocyte sedimentation rate; ETCO₂, end-tidal carbon dioxide; ETV, endoscopic third ventriculostomy; EVD, external ventricular drainage; GCS, Glasgow coma scale; HIV, human immunodeficiency virus; ICP, intracranial pressure; ICU, intensive care unit; INR, international normalised ratio; IVC, inferior vena cava; MAP, mean arterial pressure; MRI, magnetic resonance imaging; NIRS, near-infrared spectroscopy; ONSD, optic nerve sheath diameter; PbtO₂, brain tissue oxygen tension monitoring; SIADH, syndrome of inappropriate diuretic hormone secretion; TB, tuberculosis; TBI, traumatic brain injury; TBM, tuberculous meningitis; TCD, transcranial Doppler; VP, ventriculoperitoneal

Introduction

Tuberculous meningitis (TBM) is the most severe form of tuberculosis (TB) and causes critical illness from direct neurological injury or complications from the infection, treatment, or prolonged hospitalisation.

Due to serious complications, the management of TBM is complex, and includes supportive medical and neurosurgical measures. Currently there are no guidelines or standardised approach for the assessment and management of TBM. This is due to limited literature to develop an evidence-based approach, and because each TBM patient is unique. However, common themes exist, and a comprehensive proforma to guide patient assessment could be the first step towards standardised management.

Checklists can be powerful tools to focus attention¹ and their use in the medical field is growing²⁻⁴. We aimed to develop a comprehensive proforma for the assessment and management of TBM as well as a priorities checklist for the decompensating patient. The document cannot account for every scenario, but is designed to identify priorities; i.e. potentially reversible factors that contribute to morbidity and mortality. Local modifications to increase uptake and tailor use to suit local needs are encouraged.

Accompanying our proforma and checklist is the rationale for why these assessments may be important. Importantly, this article is not a guideline and does not make recommendations for care. It is not intended to replace a comprehensive ward round, nor to increase the clinical workload. Instead, it should provide a framework to highlight vital components during different stages of TBM care, with many complications overlapping throughout illness. We acknowledge that investigations and procedures will not be available at all centres.

Comprehensive proforma

The comprehensive proforma is split into initial evaluation (Table 1), daily inpatient review (Table 2), and critical care in the intensive care unit (ICU) (Table 3). However, as elements can occur at any time, the rationale is grouped by themes within sections titled "General supportive and critical care" and "Neurocritical care".

General supportive and critical care**History of present illness**

Obtaining a thorough history of the patient's signs and symptoms is paramount (Table 1 and Box 1).

Box 1. Key predictors of poor outcome in tuberculous meningitis

Increased Medical Research Council TBM disease severity⁵⁻⁸
Reduced consciousness^{5,9}
Hydrocephalus and raised ICP^{8,10,11}
Cerebral infarction^{5,12}
Seizures^{12,13}
HIV co-infection^{8,14}
Multidrug resistant, or isoniazid mono-resistant, disease¹⁵
Lower body weight⁵
Younger and older age^{5,16}

General clinical examination and monitoring

General assessment and non-invasive monitoring of vital signs may be the only tools available to guide patient management in many centres. These provide valuable information in all care settings, and are mentioned in Table 1–Table 4.

Respiratory monitoring. A change in neurological status may cause hypoxia due to airway compromise, and pulse oximetry may raise the alarm. Chest X-rays can help diagnose pulmonary TB,

Table 1. Initial evaluation.

Category	Specific question or assessment	✓
History		
	Age	
	Presenting complaints and duration (i.e., headache, irritability, vomiting, fever, neck stiffness, seizures, altered consciousness, lethargy, developmental regression, weight loss, night sweats, cough)	
	Other respiratory symptoms	
	Previous treatment for tuberculosis	
	BCG immunisation	
	History of recent TB contact	
	Other previous illnesses or comorbidities	
	If HIV positive:	
	- Date of diagnosis, treatment history, treatment adherence, recent CD4 and HIV viral load values	
General clinical examination		
	Weight and nutritional status	
	Vital signs (i.e., oxygen saturation, heart rate, blood pressure, temperature)	
	Hydration status (i.e., fluid input and output, clinical signs of dehydration)	
	Evidence of tuberculosis elsewhere (e.g., lung, lymph nodes)	
	BCG scar	
Neurological examination		
	Level of consciousness (i.e., GCS, modified for infants)	
	Pupillary exam (shape, size and reaction to light)	
	Assess for papilloedema by fundoscopy	
	Focal neurological deficits (i.e., cranial nerve palsies, hemiplegia, paraplegia, tetraplegia, urinary retention)	
	Head circumference and fontanelle in children	
CSF examination (lumbar or ventricular)		
	Opening pressure (immediately with needle insertion at lumbar puncture)	
	General appearance (i.e. colour, turbidity)	
Laboratory tests (CSF)		
	Lumbar or ventricular?	
	AFB smear	
	NAAT (e.g., GeneXpert)	
	Mycobacterial culture and drug susceptibility testing	
	White cell count (i.e., total and cell differential)	
	Protein	
	Glucose (paired with blood glucose)	
	Lactate	
Laboratory tests (blood)		
	Full blood count (i.e., haemoglobin, white blood cell count, platelets)	
	Non-specific inflammatory markers (i.e., ESR, CRP)	
	Electrolyte and renal function panel (i.e., sodium, potassium, glucose, creatinine, urea)	
	Liver function panel (i.e., ALT, AST, bilirubin)	
	Coagulation panel (i.e., INR, PTT)	
	HIV test (if positive, CD4 count and HIV viral load)	
	Serum osmolality (if hyponatraemia)	
Laboratory tests (urine)		
	Urine sodium (if hyponatraemia)	
	Urine osmolality (if hyponatraemia)	

Category	Specific question or assessment	✓
Imaging	Chest X-ray	
	Brain and/or spine (i.e. CT or MRI)	
Intracranial pressure measurement	Intermittent measurements (i.e., lumbar puncture)	
	Continuous measurements (i.e., invasive monitoring with/ without drain)	
	Assessment for communicating hydrocephalus with air encephalogram or column test	
	Non-invasive estimates of ICP	

AFB, acid-fast bacilli; ALT, alanine transaminase; AST, aspartate aminotransferase; BCG, Bacillus Calmette Guérin; CRP, C-reactive protein; CSF, cerebrospinal fluid; CT, computed tomography; ESR, erythrocyte sedimentation rate; GCS, Glasgow coma scale; HIV, human immunodeficiency virus; INR, international normalised ratio; MRI, magnetic resonance imaging; NAAT, nucleic acid amplification test; PTT, prothrombin time; TB, tuberculosis.

✓ can be selected when a proforma question has been answered, or a proforma point has been reviewed or tested.

Table 2. Daily inpatient review.

Category	Specific question or assessment	✓
General clinical examination	Weight and nutritional status (i.e., use of oral feeds, intravenous fluids, etc.)	
	Monitor for vomiting/inability to take drugs orally	
	Monitor for gastrointestinal bleeding	
	Vital signs (i.e., oxygen saturation, heart rate, blood pressure, temperature)	
	Hydration status (i.e., fluid input and output, clinical signs of dehydration, CVP, IVC ultrasound)	
Medication evaluation	Have any doses of anti-TB chemotherapy been missed?	
	Monitor for side effects from anti-TB chemotherapy	
	Check drug susceptibility testing results. Are changes to anti-TB chemotherapy required?	
	Monitor recent liver function and renal function panels for medication toxicity	
	Repeat liver function and renal function panels if toxicity concerns remain	
	Check corticosteroid dose	
Neurological examination	Schedule corticosteroid taper (i.e., when to reduce the dose)	
	Level of consciousness (i.e., GCS, modified for infants)	
	Assess for papilloedema by fundoscopy	
	Focal neurological deficits (i.e., cranial nerve palsies, hemiplegia, paraplegia, tetraplegia, urinary retention)	
	Has there been a change in examination since last review? If so, what is the suspected cause?	
Laboratory tests (blood)	Does the patient need repeat neuroimaging?	
	Repeat complete full blood count and inflammatory markers (i.e., if concern for other infection)	
	Repeat electrolyte and renal function panel (i.e., sodium, potassium, glucose, creatinine, urea) if change in hydration status	
	Repeat liver function panel (i.e., ALT, AST, bilirubin) if change in medications	
	Repeat serum osmolality if change in hydration status or new/worsening hyponatraemia	
Laboratory tests (urine)	Urine sodium if change in hydration status	
	Urine osmolality if change in hydration status	

ALT, alanine transaminase; AST, aspartate aminotransferase; CSF, cerebrospinal fluid; CVP, central venous pressure; GCS, Glasgow coma scale; IVC, inferior vena cava; TB, tuberculosis.

✓ can be selected when a proforma question has been answered, or a proforma point has been reviewed or tested.

Table 3. Critical care.

Category	Specific question or assessment	✓
General clinical examination		
	Weight and nutritional status (i.e., use of oral feeds, intravenous fluids, etc.)	
	Monitor for vomiting/inability to take drugs orally	
	Monitor for gastrointestinal bleeding	
	Vital signs (i.e., oxygen saturation, heart rate, blood pressure, temperature)	
	Hydration status (i.e., fluid input and output, clinical signs of dehydration)	
	Monitor skin for pressure damage	
Medication evaluation		
	Have any doses of anti-TB chemotherapy been missed?	
	Monitor for side effects from anti-TB chemotherapy	
	Check drug susceptibility testing results. Are changes to anti-TB chemotherapy required?	
	Monitor recent liver function and renal function panels for medication toxicity	
	Repeat liver function and renal function panels if toxicity concerns remain	
	Check corticosteroid dose	
	Schedule corticosteroid taper (i.e., when to reduce the dose)	
Vascular access		
	Is central venous access still needed?	
	Is central venous access functioning properly?	
	Are there signs/symptoms of central line-associated blood stream infection?	
	Is invasive blood pressure monitoring (arterial line) still needed?	
Urinary catheter		
	Is the urinary catheter still needed?	
	Are there signs/symptoms of catheter-associated urinary tract infection?	
Respiratory examination		
	Monitor respiratory examination	
	Monitor ventilation with end tidal CO ₂ monitoring (if available)	
	Monitor ventilation and oxygenation with arterial blood gas sampling (if available)	
	Monitor and adjust mechanical ventilation settings/modes	
	Are there signs/symptoms of ventilator-associated pneumonia?	
	Repeat chest X-ray if ventilator-associated pneumonia suspected	
	Can removal of endotracheal tube be considered?	
Neurological examination		
	Follow up neurosurgical consultation (if applicable)	
	Level of consciousness (i.e., GCS, modified for infants) – is sedation required?	
	Has there been a change in examination since last review?	
	Assess for papilloedema by fundoscopy	
	Focal neurological deficits (i.e., cranial nerve palsies, hemiplegia, paraplegia, tetraplegia, urinary retention)	
	Is repeat neuroimaging needed?	

Category	Specific question or assessment	✓
Intracranial pressure optimisation		
	Optimise head-of-bed elevation	
	Ensure appropriate sedation/analgesia	
	Check for fevers (if applicable, treat)	
	Check for appropriate blood pressure to determine cerebral perfusion pressure (if ICP is known)	
	Is continuous monitoring required (i.e., continuous parenchymal ICP or EVD)?	
	Consider non-invasive measures for evaluating ICP and brain perfusion	
Post-operative neurosurgical management		
	Monitor for changes in neurological exam	
	Monitor surgical wound for infection	
	Is repeat neuroimaging required (to check VP shunt or EVD placement)?	
	Plan for suture removal	
	If an EVD is <i>in situ</i> , check the level and check the drainage	
Laboratory tests (blood)		
	Repeat complete full blood count and inflammatory markers (i.e., if concern for other infection)	
	Repeat electrolyte and renal function panel (i.e., sodium, potassium, glucose, creatinine, urea) if change in hydration status	
	Repeat liver function panel (i.e., ALT, AST, bilirubin) if change in medications	
	Repeat serum osmolality if change in hydration status or new/worsening hyponatraemia	
Laboratory tests (urine)		
	Urine sodium if change in hydration status	
	Urine osmolality if change in hydration status	

ALT, Alanine transaminase; AST, aspartate aminotransferase; CBF, cerebral blood flow; EVD, external ventricular drain; GCS, Glasgow coma scale; ICP, intracranial pressure; TB, tuberculosis; VP, ventriculoperitoneal.

✓ can be selected when a proforma question has been answered, or a proforma point has been reviewed or tested.

Table 4. Priorities checklist for the acutely deteriorating patient with TBM.

	Reviewed
Reduced consciousness	
- Has the patient developed hydrocephalus, infarcts, cerebral venous thrombosis, or possible mass effect from tuberculomas, TB abscesses or IRIS? (consider repeat brain imaging [preferably with contrast], ICP monitoring)	
- Is the EVD or VP shunt working? (if applicable check EVD drainage, consider repeat imaging for VP shunt)	
- Is urgent neurosurgery required?	
- Have seizures been excluded?	
- Does serum glucose need correcting?	
- Does serum sodium need correcting?	
- Is there hypotension?	
Systemically unwell	
- Is supplemental oxygen required?	
- Are serum liver function tests elevated?	
- Do large urine outputs suggest hypovolaemia?	
- Is there gastrointestinal bleeding?	
- Are there signs of new infection?	

EVD, external ventricular drain; ICP, intracranial pressure; IRIS, immune reconstitution inflammatory syndrome; TBM, tuberculous meningitis; VP, ventriculoperitoneal.

Check box for each checklist question when that question has been reviewed.

and in HIV co-infected patients with *Pneumocystis jirovecii*, pneumothorax, although this is rare¹⁷.

Heart rate monitoring. Bradycardia may be caused by raised intracranial pressure (ICP) or brainstem ischaemia, and the development of tachycardia or bradycardia could indicate new infection or hypovolaemia.

Blood pressure monitoring. Blood pressure monitoring may detect septic shock or cerebral salt wasting (CSW)-associated hypotension. It may also help calculate cerebral perfusion pressure (CPP).

Temperature monitoring. Fever is associated with worse outcomes in neurocritical care and an increased one-year mortality in HIV-uninfected individuals with TBM¹. Pyrexia may indicate superimposed bacterial infection.

Medication evaluation and management

Important characteristics to monitor for anti-TB chemotherapy are described below and in Table 2.

Anti-tuberculosis chemotherapy. The optimum delivery of essential anti-TB chemotherapy is a priority, but optimal doses and administration routes are unknown¹⁸. Prompt treatment and avoidance of therapy interruptions are essential to reduce mortality, and in unconscious patients, crushed medication administered via nasogastric tube, or intravenous therapy, may be considered^{18,19}.

Anti-TB chemotherapy can change during the long duration of treatment. Regimen modifications may be necessitated by drug resistance, changes in weight, interactions with cytochrome P450-inhibiting anti-retroviral therapy (ART), and drug side effects, many of which cause and exacerbate critical illness¹⁸. Rifampicin, isoniazid, pyrazinamide and fluoroquinolones can cause liver injury; therefore, regular monitoring of alanine transaminase (ALT), aspartate aminotransferase (AST), and bilirubin is important. Optimal management of drug-induced liver injury in TBM is unknown and is currently being studied^{20,21}.

Nutrition and the gastrointestinal tract

TB causes a chronic catabolic illness and patients commonly present with weight loss or failure to thrive in children. Additionally, TBM patients are at risk of the negative consequences of the catabolic stress of critical illness²². Controlling glycaemia may be complicated by corticosteroids, which increase serum glucose. Nutrition in TBM often requires nasogastric tube placement when consciousness is reduced. Maintaining adequate nutrition is important to provide substrate for healing. Early feeding and avoidance of hypoglycaemia may improve outcomes after acute brain injury²³, but has not been studied in TBM.

Multidrug regimens, corticosteroids and anti-platelet drugs can lead to gastrointestinal intolerance and bleeding²⁴. Gastrointestinal bleeding may cause hypovolaemia and exacerbate reduced cerebral perfusion. Nausea and vomiting, common side effects of anti-TB chemotherapy, may further contribute to

hypovolaemia. Aspiration of vomited gastric contents is a risk when consciousness is impaired. See Table 1 and Table 2.

Kidney function, fluid balance, and electrolytes

Kidney function. Acute dehydration may place patients at risk for hypovolaemia and prerenal acute kidney injury. Chronic use of anti-TB chemotherapy may be nephrotoxic^{25,26}. Obtaining baseline kidney function tests, including creatinine and urea, may identify those at risk of acute or chronic kidney injury. Changes in urine output or fluid balance may precede laboratory abnormalities. See Table 1 and Table 2.

Hyponatraemia and fluid balance. Fluid balance is an important distinguishing parameter between the causes of hyponatraemia (sodium <135mmol/L). Central venous pressure (CVP) reflects the intravascular volume and helps determine hydration status. CVP can be measured continuously or intermittently from a central venous catheter most often placed in the internal jugular vein or femoral vein if they are available. CSW is often characterised by high volume urine output, hypovolaemia with low CVP, clinical signs of dehydration (dry mucous membranes, delayed capillary refill time, tachycardia, and hypotension) and haemoconcentrated laboratory parameters (elevated haematocrit, haemoglobin or urea)²⁷. Conversely, the syndrome of inappropriate anti-diuretic hormone (SIADH) lacks a high volume urine output, and patients are usually euvoelaemic with a normal CVP, and no clinical signs of dehydration²⁷. Fluid balance charts may help identify these fluid shifts. Despite these distinguishing features, CSW and SIADH can be difficult to diagnose and other laboratory tests, such as serum and urinary osmolality and urinary sodium, may help further identify the aetiology, which is critical due to their divergent management approaches.

Hypokalaemia. Hypokalaemia may be a result of drugs or of poor nutrition, whereas hyperkalaemia may be due to hyponatremia either directly from TB or from withdrawal of corticosteroids. Monitoring low serum potassium and replacement requires an environment capable of close monitoring. The use of hypertonic saline, fludrocortisone and acetazolamide, individually or in combination, may rapidly shift electrolytes.

Risks of prolonged critical care admission

Pressure ulcers are common in immobile individuals requiring prolonged care. Nosocomial infections occur due to changes in patients' immune system and placement of foreign objects, such as a central venous line, arterial line, urinary catheter or endotracheal tube. Deep vein thrombosis is a risk of prolonged critical care admission. Urinary tract infections may occur secondary to urinary catheters. Further, impaired consciousness may require prolonged intubation and mechanical ventilation²⁸, which may be associated with a higher risk for gastrointestinal haemorrhage, septicemia and pressure ulcers²⁹. Prolonged recovery and slow ventilator wean may involve tracheostomy placement.

Neurocritical care

In addition to general and critical care management, specific neurocritical care may assist in TBM management.

Neurological examination

Level of consciousness. The Glasgow coma scale (GCS) assesses level of consciousness. It can be confounded by intubation, sedatives, and pre-existing neurological conditions^{18,30}. For paediatrics, modified GCS versions have been developed, but are used variably³⁰. Decreased GCS (<15) could be due to irreversible neurological injury and also reversible factors including electrolyte disturbances, raised ICP, seizures, and medication. Consequently, the GCS one week post-admission may be a stronger prognostic marker³¹. A deteriorating GCS may signal worsening hydrocephalus, poorly controlled ICP, and progressive ischaemia.

Focal neurological deficits. Palsies commonly involve cranial nerves II, III and VI and can denote nerve arachnoiditis, ischaemia, a mass lesion, or hydrocephalus³². Motor weakness and abnormalities may be due to ischaemia, infarction or brain shift.

Cranial examination. An enlarging head may signify subacute or chronic development of hydrocephalus in young children if their sutures are not fused.

General monitoring in neurocritical care

Blood pressure and cerebral perfusion. Maintaining normotension is important for adequate CPP, defined as mean arterial pressure (MAP) minus ICP, which reflects the pressure gradient that drives cerebral blood flow (CBF)³³. Current treatment guidelines in traumatic brain injury (TBI) recommend maintaining age-appropriate CPP and MAP^{33–36}. Hydration status and overall fluid balance, often compromised by CSW or poor oral intake, can affect MAP and subsequently CPP. TBM-specific goals for CPP and MAP are not established.

Ventilation and oxygenation. Oxygen administration may increase cerebral oxygenation and possibly reverse brain hypoxia³⁷. Oxygen and carbon dioxide (CO₂) levels also affect CBF and ICP. Decreased arterial oxygen and increased CO₂ both dilate cerebral vessels, which may increase cerebral blood volume and ICP³⁸. Conversely, aggressive hyperventilation may constrict cerebral vessels and cause ischaemia from decreased CBF³⁹. Therefore, tight control of end tidal carbon dioxide (ETCO₂) is crucial to control raised ICP but avoid ischaemia and is an important parameter in TBI management guidelines, although the target level for TBM is unknown^{34,39}. Both oxygenation and ventilation can be monitored noninvasively with pulse oximetry and ETCO₂ or invasively with arterial blood gases.

Temperature. Hyperthermia may increase cerebral metabolic rate and CBF, which can further increase ICP in a swollen brain⁴⁰. Induced hypothermia is experimentally neuroprotective but is associated with poorer outcomes in TBI^{34,35,39}. The target temperature in TBM is not established.

Head-of-bed elevation. Elevating the head end of a bed lowers ICP through improved venous drainage and extracranial shift of cerebrospinal fluid (CSF). However, the MAP may also

fall. In non-TBM pathology, studies suggest a beneficial or non-detrimental role of head-of-bed elevation to 30°^{41–43}.

Neuroimaging

Infarcts may not be visible on admission computed tomography (CT) scans^{44,45}, but diffusion-weighted magnetic resonance imaging (MRI) is sensitive to acute/evolving infarcts^{46,47}. Follow-up imaging is important to detect new infarcts^{44,45,48} and, although there are little data on the temporal profile of infarct development, patients appear to be at greatest risk during the acute phase (first month).

Hydrocephalus is the commonest cause of increased ICP in TBM^{45,49}. The communicating nature of hydrocephalus has implications for the safety of lumbar punctures and the method of treatment. However, communication cannot be determined from standard imaging⁵⁰ and lumbar exudate can confound determination of the level of CSF obstruction. ICP cannot be estimated from ventricular size alone on imaging⁴⁸. Hydrocephalus may persist or develop *de novo* over the first six months after treatment initiation; therefore, repeat neuroimaging may be warranted^{10,51}.

Tuberculomas and TB abscesses occasionally complicate the management of TBM if they cause local mass effect⁵² and precipitate CSF obstruction, focal deficits or seizures^{45,48,53,54}. During treatment, paradoxical enlargement of established tuberculomas or development of new tuberculomas may necessitate follow-up imaging^{45,53,55–57}.

Repeat neuroimaging can be used to monitor disease progression, evaluate deteriorating patients, and confirm the placement of a ventriculoperitoneal (VP) shunt or an external ventricular drain (EVD). The ideal timing of follow up imaging is unclear in stable patients.

Raised intracranial pressure

Raised ICP is a key factor precipitating adverse outcomes. Firstly, it reduces CPP and exacerbates existing cerebral ischaemia due to vasculitis. Secondly, generalised or compartmentalised increased ICP causes brain shift and consequent neural injury. ICP monitoring is useful; non-invasive monitoring techniques may be used when gold-standard invasive monitoring is unavailable. New monitoring techniques have potential for improving patient care, yet these are not widely available. Important points for the monitoring and optimisation of ICP are shown in Table 3 and Table 4.

Non-invasive intracranial pressure measurement and monitoring

Fundoscopy

Optic disc swelling can indicate raised ICP. However, fundoscopy is challenging to perform and highly operator dependent, and the development of papilloedema can be delayed.

Optic nerve sheath diameter ultrasound

Changes in optic nerve sheath diameter (ONSD) due to raised ICP occur rapidly and can be measured using ultrasound⁵⁸.

ONS ultrasound is quick, easy, and reproducible, and correlates with ICP^{59,60}, although evidence for its use in TBM is limited^{61–63}.

Compromised cerebral perfusion

Various neuromonitors have been used in non-TBM pathologies, to detect ischaemic brain injury^{64–66}. These measure various facets of brain perfusion and each have strengths and limitations.

Transcranial Doppler ultrasound

Transcranial Doppler (TCD) can be used to measure flow velocity in basal vessels and detect vasculopathy; however, it may not detect mild-moderate ICP changes, is limited to flow in the major cerebral vessels, and is technically challenging^{18,67}.

Non-invasive cerebral oxygenation monitoring: near-infrared spectroscopy. Near-infrared spectroscopy (NIRS) is a non-invasive monitor that uses optical technology to continuously assess brain oxygenation^{68,69}. NIRS is limited by superficial penetration of cortex, distortion by the skull, CSF and oedema^{68,70,71} and poor long term monitoring.

Invasive cerebral oxygenation monitoring: partial pressure of brain tissue oxygen tension. The partial pressure of brain tissue oxygen tension (PbtO₂) monitor is a thin parenchymal catheter that offers continuous monitoring of brain oxygenation^{66,72}. Normal values have not been established; however, the risk of poor outcome increases with PbtO₂ <20mmHg⁷³, especially <10mmHg^{68,72,74,75}.

Invasive intracranial pressure measurement and monitoring. CSF opening pressure may be measured from the ventricles with an EVD or via lumbar puncture (when safe)⁷⁶. CSF drainage allows simultaneous ICP monitoring and treatment. Continuous monitoring is possible with a parenchymal probe.

Post-operative neurosurgery management. This includes wound review, suture removal, and clinical monitoring for signs of treatment failure. Repeat imaging can check treatment success. EVDs must be carefully managed to avoid life-threatening complications (infection and overdrainage-related intracranial haemorrhage). VP shunts are permanent and therefore complication rates must be viewed over the full lifetime of the patient.

Management of acutely decompensating patients

Causes for acute neurological decompensation include raised ICP, metabolic disturbances (i.e., hyponatremia, hypoglycaemia), stroke (ischaemic or haemorrhagic) and seizures. Table 4 outlines a priorities-based checklist approach to the acutely decompensating patient. The rationale for this checklist is described below, unless already discussed.

Seizures

Clinical and subclinical seizures can increase ICP due to the increased cerebral metabolic demand and resultant increased

CBF. Hydrocephalus, infarcts, tuberculomas, and electrolyte imbalance can all precipitate seizures. Anti-convulsants that induce cytochrome P450 enzymes, or are susceptible to enzyme induction by rifampicin, may complicate management.

Hyperosmolar treatment

Intravenous administration of a hyperosmolar solution creates an osmotic gradient, removing water from the brain and decreasing ICP⁷⁷. Hypertonic saline may lower ICP faster, further, and for longer than mannitol¹⁸; however, no trials have directly compared these agents in TBM.

Raised intracranial pressure surgical management

Hydrocephalus

VP shunting has long been standard practice for hydrocephalus but may be associated with complications^{78,79}. EVD may be used for temporary drainage of CSF, and to assess the benefit of a VP shunt in patients with an altered sensorium. With endoscopic third ventriculostomy (ETV), CSF is drained internally by connecting the ventricles with the subarachnoid space via a stoma in the floor of the third ventricle. ETV is particularly challenging in TBM and experience is required^{80,81}.

Mass lesions

Surgical excision for tuberculomas is uncommon but may be indicated depending on their size, location, expansion, and clinical consequences. Surgery is more commonly needed for TB abscesses (drainage and/or excision).

Cerebral venous thrombosis

Cerebral venous thrombosis is an unusual cause of acute neurological deterioration in TBM, but has been described^{82,83}.

Data availability

No data are associated with this article.

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The authors have attempted to streamline the management of TBM and its complications in a step wise fashion and is a welcome step. However, there is nothing new in what they have mentioned and is practised in the countries where the disease is endemic. I am fine with summary for management except for a small comment on table 1 and is as under:

Table 1:

- Assessment for communicating hydrocephalus with air encephalogram or column test is obsolete and is only of historical relevance and should be deleted.

Is the rationale for the Open Letter provided in sufficient detail?

Yes

Does the article adequately reference differing views and opinions?

Yes

Are all factual statements correct, and are statements and arguments made adequately supported by citations?

Partly

Is the Open Letter written in accessible language?

Yes

Where applicable, are recommendations and next steps explained clearly for others to follow?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Imaging

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 22 Jan 2020

Joseph Donovan, Oxford University Clinical Research Unit, Centre for Tropical Medicine, Ho Chi Minh City, Vietnam

Dear Dr Rakesh K. Gupta,,

Thank you for reviewing our open letter "Checklists to guide the supportive and critical care of tuberculous meningitis".

Please find below our point by point responses.

1. The authors have attempted to streamline the management of TBM and its complications in a step wise fashion and is a welcome step. However, there is nothing new in what they have mentioned and is practiced in the countries where the disease is endemic. I am fine with summary for management except for a small comment on table 1 and is as under:

Table 1: Assessment for communicating hydrocephalus with air encephalogram or column test is obsolete and is only of historical relevance and should be deleted.

Thank you for your comment. However, we disagree that this does not offer anything new; our proforma is the first comprehensive patient assessment tool for tuberculous meningitis. No other checklist exists to allow a priority-based approach to a deteriorating patient in this disease. Whilst the information included in the checklist and proforma may be accepted knowledge, or available elsewhere, our presentation of this information in proforma and checklist formats aim to support clinical assessment, and highlight vital components, during clinical care.

2. Table 1: Assessment for communicating hydrocephalus with air encephalogram or column test is obsolete and is only of historical relevance and should be deleted.

We note, but disagree with, the reviewer's comment on air encephalography and column tests.

These are used as standard approaches in at least two big centres that publish on TB meningitis, based on published data, so this is hardly historical nor obsolete.

There is no current technology, apart from invasive methods, that have been shown to safely and reliably distinguish between communicating and non-communicating hydrocephalus. This is based on published studies. We know that some centres do not try to distinguish and therefore have higher rates of surgical procedures - VP shunting and endoscopy. However, with medical management, most patients can avoid those surgical procedures - this was published by Johan Schoeman many years ago and the results are as relevant today as they were then. But this of course depends on being able to do lumbar punctures safely, which may be risky for the 15-20% of patients that may have non-communicating hydrocephalus. To our knowledge, there has been no paper showing the safety and reliability of any imaging to confirm the communicating nature of hydrocephalus in TBM.

So the reviewer's comment is not evidence-based and we are comfortable that our manuscript reflects published data.

Competing Interests: No competing interests were disclosed.

Reviewer Report 03 December 2019

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Abdu Kisekka Musubire 

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The authors attempt to answer a very relevant question with scarce data. The article is timely and very important. I have the following concerns:

Box 1 page 3: Define the extremes of ages for the readers

Under respiratory monitoring: page 3
Pneumothorax is rare in PJP

Swiss Med Wkly. 2019 Oct 3;149:w20130. Imaging patterns of *Pneumocystis jirovecii* pneumonia in HIV-positive and renal transplant patients - a multicentre study. Christe A1, Walti L2, Charimo J1, Rauch A2, Furrer H2, Meyer A3, Huynh-Do U3, Heverhagen JT4, Mueller NJ5, Cavassini M6, Mombelli M6, van Delden C7, Frauenfelder T8, Montet X9, Beigelman-Aubry C10, Arampatzis S11, Ebner L3¹.

Indian J Crit Care Med. 2015 Jan;19(1):34-7. Paroxysmal sympathetic hyperactivity in neurological critical care. Verma R1, Giri P1, Rizvi I1².

Still on page 3, the roles for Heart rate monitoring are presented in a limited way
Tachycardia is common and directly associated with TBM.

West Indian Med J. 2015 Dec;64(5):543-547. Epub 2016 Apr 29. Paroxysmal Sympathetic Hyperactivity in a Child with Tuberculous Meningitis A Case Study and Review of Related Literature. Xu Y1, Wan L2, Ning J2, Guo W2, Ren L2³.

Int J Infect Dis. 2010 Jul;14(7):e586-91. Tuberculous meningitis in HIV-infected patients in Brazil: clinical and laboratory characteristics and factors associated with mortality. Croda MG1, Vidal JE, Hernández AV, Dal Molin T, Gualberto FA, de Oliveira AC⁴.
Author information

Still under temperature monitoring. The reader is interested in clues as to how to differentiate the temperature of TBM from super imposed infection?

Table 1 page 4: Relevance of the previous BCG scar?
I propose that the tests be separated in to those with high utility like gene expert, low CSF glucose from those with low utility
like AAFB, culture. Imaging must always be contrasted unless contraindicated

Table 2: The part on monitoring for GIT bleeding, what is the practical way of doing this? Not so much information about its relevance in TBM patients.

If possible the authors should expound on how to suspect superimposed infections

Table 4 page 7. In a rapidly deteriorating patient with TBM, paradoxical reactions like IRIS needs to be mentioned especially in HIV positive patients. And cerebral venous thrombosis(BMJ Case Rep. 2013 Aug 5;2013. pii: bcr2013009892⁵. Indian J Pediatr. 2019 Apr;86(4):371-378. Epub 2019 Jan 9⁶.

Risks of prolonged hospital admission should include DVT.

Page 9 Last paragraph under neuroimaging needs a reference.

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Is the rationale for the Open Letter provided in sufficient detail?

Yes

Does the article adequately reference differing views and opinions?

Yes

Are all factual statements correct, and are statements and arguments made adequately supported by citations?

Partly

Is the Open Letter written in accessible language?

Yes

Where applicable, are recommendations and next steps explained clearly for others to follow?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Internal Medicine

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 22 Jan 2020

Joseph Donovan, Oxford University Clinical Research Unit, Centre for Tropical Medicine, Ho Chi Minh City, Vietnam

Dear Dr Abdu Kisekka Musubire,

Thank you for reviewing our open letter "Checklists to guide the supportive and critical care of tuberculous meningitis".

Please find below our point by point responses.

1. Box 1 page 3: Define the extremes of ages for the readers

'Older age' is based upon a Cox regression model for 9-month survival in HIV uninfected adults with TBM.¹ An increase in age (per +10 years) gave a hazard ratio of 1.24 (95% CI 1.15-1.34, $p < 0.001$). In this HIV uninfected group, median age was 40 years (IQR 27-56 years). Regarding 'younger age', in a study of 214 children with TBM (mean age at presentation 4.1 years), 49 patients (23%) died.² 63% of deaths were in age 5 or younger; however this age group is represented more frequently. Defining 'extremes of age' in adults is challenging; these are based on age ranges of individual studies. In paediatrics it is broadly accepted that most cases occur in children < 5yrs, and therefore more deaths are likely in that age range.

2. Under respiratory monitoring: page 3, Pneumothorax is rare in PJP

We have added here that this is rare and referenced this³

3. Still on page 3, the roles for Heart rate monitoring are presented in a limited way

Tachycardia is common and directly associated with TBM.

Tachycardia is common with many medical conditions. In our article we have tried to focus on critical care of TBM. Whilst it clearly can occur, we do not feel paroxysmal sympathetic hyperactivity is sufficiently associated with TBM to include here. In the first cited study,⁴ there was only one case of TBM associated with paroxysmal sympathetic hyperactivity. The second cited study⁵ notes it is rare in TBM. We note the reference describing tachycardia being associated with mortality in a HIV infected cohort.⁶

4. Still under temperature monitoring. The reader is interested in clues as to how to differentiate the temperature of TBM from super imposed infection?

This is a valid point, and we are not suggesting there are clues to differentiate these. We wish to note that fever in TBM may not only be due to TBM disease. Resolution of TBM-associated fever, followed by the development of new fever, may indicate new infection.

5. Table 1 page 4: Relevance of the previous BCG scar?

BCG vaccine protects against meningeal and miliary TB in infants⁷

6. I propose that the tests be separated in to those with high utility like gene expert, low CSF glucose from those with low utility like AAFB, culture.

We feel the value of these tests will vary by site, and have therefore kept these listed together.

7. Imaging must always be contrasted unless contraindicated

This is a good point. However the addition of contrast may not always be possible. We have added 'preferably with contrast' to table 4

8. Table 2: The part on monitoring for GIT bleeding, what is the practical way of doing this? Not so much information about its relevance in TBM patients.

Recommending how to practically monitor for GI bleeding goes beyond the scope of this article. This adverse event is relevant given frequent dexamethasone use in TBM, and the increasing evidence base for aspirin⁸

9. If possible the authors should expound on how to suspect superimposed infections.

Although this is an important component to TBM care, we feel expanding on this is beyond the scope of this article

10. Table 4 page 7. In a rapidly deteriorating patient with TBM, paradoxical reactions like IRIS needs to be mentioned especially in HIV positive patients. And cerebral venous thrombosis(BMJ Case Rep. 2013 Aug 5;2013. pii: bcr2013009892⁵. Indian J Pediatr. 2019 Apr;86(4):371-378. Epub 2019 Jan 9⁶.

We have now specifically mentioned IRIS and cerebral sinus thrombosis in table 4.

In the supporting document we have added the sentence 'Cerebral venous thrombosis is an unusual cause of acute neurological deterioration in TBM, but has been described' with references.

11. Risks of prolonged hospital admission should include DVT.

We have added 'Deep vein thrombosis is a risk of prolonged critical care admission'

12. Page 9 Last paragraph under neuroimaging needs a reference.

We have discussed the repeat imaging with references in the preceding paragraphs, and repeat imaging after placing hardware in the brain is standard neurosurgical practice

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Competing Interests: No competing interests were disclosed.

Xpert MTB/RIF Ultra versus Xpert MTB/RIF for the diagnosis of tuberculous meningitis: a prospective, randomised, diagnostic accuracy study

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Summary

Background Xpert MTB/RIF Ultra (Xpert Ultra) might have higher sensitivity than its predecessor, Xpert MTB/RIF (Xpert), but its role in tuberculous meningitis diagnosis is uncertain. We aimed to compare Xpert Ultra with Xpert for the diagnosis of tuberculous meningitis in HIV-uninfected and HIV-infected adults.

Methods In this prospective, randomised, diagnostic accuracy study, adults (≥ 16 years) with suspected tuberculous meningitis from a single centre in Vietnam were randomly assigned to cerebrospinal fluid testing by either Xpert Ultra or Xpert at baseline and, if treated for tuberculous meningitis, after 3–4 weeks of treatment. Test performance (sensitivity, specificity, and positive and negative predictive values) was calculated for Xpert Ultra and Xpert and compared against clinical and mycobacterial culture reference standards. Analyses were done for all patients and by HIV status.

Findings Between Oct 16, 2017, and Feb 10, 2019, 205 patients were randomly assigned to Xpert Ultra (n=103) or Xpert (n=102). The sensitivities of Xpert Ultra and Xpert for tuberculous meningitis diagnosis against a reference standard of definite, probable, and possible tuberculous meningitis were 47.2% (95% CI 34.4–60.3; 25 of 53 patients) for Xpert Ultra and 39.6% (27.6–53.1; 21 of 53) for Xpert (p=0.56); specificities were 100.0% (95% CI 92.0–100.0; 44 of 44) and 100.0% (92.6–100.0; 48 of 48), respectively. In HIV-negative patients, the sensitivity of Xpert Ultra was 38.9% (24.8–55.1; 14 of 36) versus 22.9% (12.1–39.0; eight of 35) by Xpert (p=0.23). In HIV co-infected patients, the sensitivities were 64.3% (38.8–83.7; nine of 14) for Xpert Ultra and 76.9% (49.7–91.8; ten of 13) for Xpert (p=0.77). Negative predictive values were 61.1% (49.6–71.5) for Xpert Ultra and 60.0% (49.0–70.0) for Xpert. Against a reference standard of mycobacterial culture, sensitivities were 90.9% (72.2–97.5; 20 of 22 patients) for Xpert Ultra and 81.8% (61.5–92.7; 18 of 22) for Xpert (p=0.66); specificities were 93.9% (85.4–97.6; 62 of 66) and 96.9% (89.5–91.2; 63 of 65), respectively. Six (22%) of 27 patients had a positive test by Xpert Ultra after 4 weeks of treatment versus two (9%) of 22 patients by Xpert.

Interpretation Xpert Ultra was not statistically superior to Xpert for the diagnosis of tuberculous meningitis in HIV-uninfected and HIV-infected adults. A negative Xpert Ultra or Xpert test does not rule out tuberculous meningitis. New diagnostic strategies are urgently required.

Funding Wellcome Trust and the Foundation for Innovative New Diagnostics.

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Introduction

Mycobacterium tuberculosis kills more people each year than any other infectious disease.¹ Tuberculous meningitis is the most severe form of tuberculosis, resulting in death or disability in approximately half of those it affects.² Delayed diagnosis and treatment are strongly linked to poor outcomes, a situation exacerbated by conventional diagnostic tests for *M. tuberculosis*, which are insufficiently sensitive. Cerebrospinal fluid (CSF) smear microscopy is widely available, yet sensitivity following Ziehl–Neelsen staining is often low.³ Culture of *M. tuberculosis* takes several weeks and cannot guide initial treatment decisions.

Xpert MTB/RIF (Xpert; Cepheid, Sunnyvale, CA, USA) offered a breakthrough in tuberculosis diagnostics: a rapid,

highly sensitive nucleic acid amplification test (NAAT) with additional rifampicin susceptibility testing. Xpert uses a hemi-nested real-time PCR assay to detect and amplify an *M. tuberculosis*-specific sequence of the bacterial *rpoB* gene.⁴ Xpert is valuable when positive, yet it is insufficiently sensitive to exclude tuberculous meningitis when negative. Meta-analyses of the diagnostic performance of Xpert for tuberculous meningitis showed pooled sensitivities of 79.5–80.5% compared with mycobacterial culture and specificities of 98.6–98.8% for *M. tuberculosis* detection in CSF.^{5,6} However, sensitivity is affected by the volume of CSF tested, whether CSF centrifugation was done before testing,⁷ and the choice of diagnostic gold standard. Use of a clinical reference standard, wherein not

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Research in context

Evidence before this study

We searched PubMed Central for all studies or reports of Xpert MTB/RIF Ultra (Xpert Ultra) for the diagnosis of tuberculous meningitis, using the terms “tuberculous meningitis” OR “TB meningitis” OR “extrapulmonary” AND “Xpert Ultra”, up to Sept 14, 2019. No language restrictions were applied. Our search returned five relevant publications that included patients undergoing Xpert Ultra testing of cerebrospinal fluid (CSF) for diagnosis of tuberculous meningitis. One study did Xpert Ultra and Xpert testing on thawed pre-centrifuged cryopreserved CSF from 23 patients screened for a trial of treatment for HIV co-infected cryptococcal meningitis. Sensitivities were 69.6% (16 patients) for Xpert Ultra and 43.5% (ten patients) for Xpert, compared with a reference standard of definite or probable tuberculous meningitis. Of 21 cases positive by Xpert Ultra, Xpert Ultra was the only positive mycobacterial test in eight cases, suggesting Xpert Ultra might detect cases of tuberculous meningitis below the threshold of detection of other confirmatory mycobacterial tests. Subsequent to this study, WHO recommended Xpert Ultra replace Xpert in all settings.

Three studies (in four to 43 patients) compared Xpert Ultra and Xpert for diagnosis of extrapulmonary tuberculosis in patients with suspected tuberculous meningitis. The study containing the largest patient group used at least 3 mL of uncentrifuged CSF for testing. Sensitivities were 44.2% (19 of 43) for Xpert Ultra and 18.6% (eight of 43) for Xpert ($p=0.01$). Although the reported sensitivity of Xpert Ultra was higher than that of Xpert when testing bacteriologically confirmed tuberculous meningitis (86.4% [19 of 22] vs 36.4% [eight of 22]; $p=0.001$), cases positive only by nucleic acid amplification tests were included in the reference standard. Of the 43 cases, all were smear negative and only three were culture positive. A case series of 11 patients (two of whom were HIV co-infected) with definite or probable tuberculous meningitis who underwent CSF testing found positive tests in seven patients with Xpert Ultra and four with Xpert. No randomised comparison of the two methods has been done to date, and the role of Xpert Ultra in tuberculous meningitis diagnosis remains controversial.

Added value of this study

To our knowledge, this study in 205 individuals, including 108 treated for tuberculous meningitis, is the first randomised

comparison of Xpert Ultra and Xpert. We provide information about the post-treatment performance of both tests.

Tuberculous meningitis is associated with very low numbers of bacteria in CSF; therefore, the CSF volume tested is a crucial determinant of the diagnostic performance of tests that directly detect bacteria or their nucleic acids. We thus chose to collect and test large volumes (6 mL) of CSF and randomly assign patients to Xpert Ultra or Xpert CSF testing, rather than halving the CSF sample for use in two concurrent tests.

This approach maximised the CSF volumes tested, providing a better estimate of diagnostic performance and mimicking clinical practice in which only one molecular test would normally be done. We showed that Xpert Ultra was not superior to Xpert when compared against either clinical or mycobacterial culture reference standards. Specificity of tuberculous meningitis diagnosis was not reduced with Xpert Ultra when compared against a clinical reference standard—an important finding, given the reduction in specificity previously described with Xpert Ultra testing of sputum samples for pulmonary tuberculosis. In pulmonary tuberculosis, Xpert Ultra has shown superior sensitivity to Xpert when testing sputum samples with low bacillary load. HIV-uninfected patients with tuberculous meningitis are considered to have lower CSF bacillary loads than those with HIV co-infection, and in the HIV-uninfected group of our study, Xpert Ultra had a higher sensitivity than Xpert against the clinical and mycobacterial culture reference standards, although the differences were not significant.

Implications of all the available evidence

Xpert Ultra showed a modest increase in sensitivity compared with Xpert for the diagnosis of tuberculous meningitis and after the start of antituberculosis treatment, but the differences were not significant. These differences appeared to be greater in HIV-uninfected than in HIV-infected patients with tuberculous meningitis, which suggests Xpert Ultra might perform better than Xpert when bacterial numbers are very low. However, Xpert Ultra's negative predictive value (61.1%) remains too low to be used to rule out tuberculous meningitis. The search must continue for a better diagnostic test for tuberculous meningitis.

all cases are microbiologically confirmed, results in reduced Xpert sensitivity. Standardised diagnostic criteria proposed by Marais and colleagues⁶ are frequently used to compare tuberculous meningitis diagnostic test performance, yet inconsistency in inclusion of some or all of definite, probable, and possible cases in the reference standard limits study comparison.

Xpert MTB/RIF Ultra (Xpert Ultra) aims to improve the sensitivity of tuberculosis diagnosis and enhance rifampicin resistance identification. A larger reaction chamber,

plus incorporation of two different multicopy amplification targets (IS6110 and IS1081) intend to reduce the limit of detection of bacterial colony-forming units.⁹ Adaptation of molecular probes and testing approach are designed to differentiate between silent mutations and mutations conferring resistance.¹⁰ A prospective multicentre study in adults with pulmonary tuberculosis showed that Xpert Ultra had a higher diagnostic sensitivity than Xpert: 63% versus 46% in smear-negative, culture-positive sputum samples ($n=135$) and 90% versus

77% in culture-positive sputum samples from HIV co-infected individuals ($n=115$).¹¹ However, no improvement in sensitivity was seen among HIV-uninfected individuals (91% for Xpert Ultra vs 90% for Xpert), and Xpert Ultra specificity was lower than Xpert (96% vs 98%).

In 2017, Bahr and colleagues¹² reported the first published study of Xpert Ultra for the diagnosis of tuberculous meningitis, testing CSF obtained during screening for a study of HIV co-infected cryptococcal meningitis. In 23 HIV co-infected patients with definite or probable tuberculous meningitis, pre-centrifuged cryopreserved CSF was thawed and retrospectively tested with Xpert Ultra. In that study, the sensitivities were 69.6% (16 of 23) for Xpert Ultra and 43.5% (ten of 23) for Xpert when compared against a reference standard of definite or probable tuberculous meningitis. Subsequently, Wang and colleagues¹³ assessed Xpert Ultra for the diagnosis of extrapulmonary, paucibacillary tuberculosis, including 43 CSF samples from HIV-uninfected adults with suspected tuberculous meningitis. At least 3 mL of uncentrifuged CSF was tested by both Xpert Ultra and Xpert, with Xpert Ultra showing higher diagnostic sensitivity than Xpert (44.2% [19 of 43] vs 18.6% [eight of 43]; $p=0.01$) against a reference standard of definite, probable, and possible tuberculous meningitis. Smear or culture diagnoses were infrequent (none by smear and three by culture), and evaluation of sensitivity in bacteriologically confirmed cases relied heavily on cases positive only by Xpert Ultra or Xpert. Two other studies of Xpert Ultra testing of extrapulmonary samples included 16 and four CSF samples of suspected tuberculous meningitis, respectively,^{14,15} of which two (13%) of 16 (both culture negative) and three (75%) of four (all culture positive) were positive by Xpert Ultra. In 2019, Chin and colleagues¹⁶ described 11 patients with definite or probable tuberculous meningitis (two of whom were HIV co-infected) who underwent CSF testing by both Xpert Ultra and Xpert. Tests were positive for seven (64%) patients with Xpert Ultra and four (36%) patients with Xpert.

To date, studies comparing Xpert Ultra with Xpert for tuberculous meningitis diagnosis have been small, involved retrospective testing, and have included few patients with microbiologically confirmed tuberculous meningitis. However, WHO now recommend replacement of Xpert with Xpert Ultra in all settings.¹⁷ We therefore did a large, randomised, prospective comparison of the two diagnostic tests to better define the role of Xpert Ultra in the diagnosis of tuberculous meningitis.

Methods

Study design and participants

We did a prospective, randomised, observational study to compare the performances of Xpert Ultra and Xpert for the diagnosis of tuberculous meningitis. In this study, diagnostic tests were randomly allocated to patients and diagnostic performances were evaluated; as this study

was not a randomised clinical trial, it was not registered as such. Patients aged 16 years or older with suspected tuberculous meningitis based on clinical and CSF findings (clear or mildly cloudy CSF, plus >5 days of symptoms consistent with tuberculous meningitis⁸ or low CSF glucose or raised CSF lactate concentrations) at The Hospital for Tropical Diseases (Ho Chi Minh City, Vietnam) were eligible for enrolment. Patients were excluded if lumbar puncture was contraindicated or informed consent was not given by the patient or by a relative if the patient did not have capacity. The study was approved by the Hospital for Tropical Diseases and the Oxford Tropical Research Ethics Committee.

Procedures

At baseline, patients were randomly assigned to undergo Xpert Ultra or Xpert testing of CSF obtained by lumbar puncture. A randomisation list was generated using a program written in R version 3.4. A CSF volume of 6 mL was used for mycobacterial tests; if less than 6 mL was taken, the tests were still done, with all CSF volumes recorded. CSF was centrifuged at 3000 g for 15 minutes. The supernatant was removed and the deposit was resuspended in the remaining 500 μ L. 100 μ L was used for Ziehl–Neelsen smear, 200 μ L for mycobacterial culture (mycobacteria growth indicator tube [MGIT]), and 200 μ L for either Xpert Ultra or Xpert. Ziehl–Neelsen smear, MGIT, and Xpert were done following standard procedures, as previously described.¹⁸ When MGIT testing was positive, phenotypic drug susceptibility testing was done by a Bactec MGIT SIRE kit (Becton, Dickinson; Franklin Lakes, NJ, USA) as previously described.¹⁸ Xpert Ultra and Xpert testing were done by laboratory technicians (DDAT, VTMD, TPQ) masked to the patient's clinical characteristics.

At the end of the trial, all patients received a final diagnosis of definite, probable, possible, or not tuberculous meningitis according to the published uniform case definition for tuberculous meningitis clinical research.⁹ Disease severity was assessed by the Medical Research Council tuberculous meningitis grade. Patients with probable and possible diagnoses could be reassigned to not tuberculous meningitis if the treating clinician did not consider the final diagnosis to be tuberculous meningitis and the patient recovered without antituberculosis chemotherapy. Patients were treated following local and national guidelines. Repeat testing was done according to the initial randomisation group on routine follow-up CSF taken 3–4 weeks after treatment initiation for those treated for tuberculous meningitis. Final reference standard diagnoses were assigned without the Xpert Ultra or Xpert result contributing to the final diagnosis.

HIV testing was not mandatory for this study and was done when clinically indicated. All patients reported as HIV negative had a negative HIV test at baseline. Of

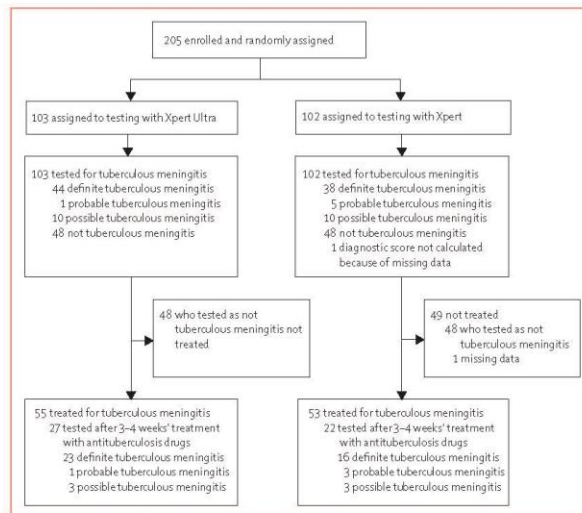


Figure 1: Trial profile
Xpert Ultra–Xpert MTB/RIF Ultra, Xpert–Xpert MTB/RIF.

those reported HIV positive, either a test was positive at baseline or previous HIV positivity was recorded.

Outcomes

Diagnostic performances (sensitivity, specificity, and positive and negative predictive values) of Xpert Ultra, Xpert, smear, and MGIT culture were compared against the clinical reference standards of definite, probable, and possible tuberculous meningitis, definite and probable tuberculous meningitis, and definite tuberculous meningitis. Additionally, diagnostic performances of Xpert Ultra and Xpert were compared against a mycobacterial reference standard (MGIT culture). Both clinical and microbiological reference standards were used because of the absence of a single gold-standard test for tuberculous meningitis. A post-hoc analysis of CSF volume influencing the likelihood of a positive Xpert Ultra or Xpert test was done, where CSF volume used for mycobacterial testing was divided into three categories: more than 5 mL, 2–5 mL, and no more than 2 mL, consistent with CSF volume intervals in a previous study.¹⁸ In addition, the diagnostic performances of Xpert Ultra and Xpert were evaluated by HIV status, given HIV co-infection has been shown to improve Xpert sensitivity for the diagnosis of tuberculous meningitis.¹⁸ Test performances after 3–4 weeks of anti-tuberculosis treatment were also evaluated against the uniform case definition for tuberculous meningitis in patients who received antituberculosis chemotherapy. Diagnostic performances of Xpert Ultra and Xpert for

rifampicin resistance prediction were evaluated against phenotypic drug-susceptibility testing of MGIT-positive cases. An exploratory analysis comparing Xpert and Xpert Ultra for semi-quantification of CSF bacterial numbers into high, medium, low, or very low categories (and trace for Xpert Ultra) was also done.

Statistical analysis

Test performance measures (sensitivity, specificity, and positive and negative predictive values) with associated Wilson CIs were calculated for Xpert Ultra and Xpert and compared with those for Ziehl–Neelsen smear and MGIT using the χ^2 test.

The study by Bahr and colleagues suggested Xpert Ultra was 25% more sensitive than Xpert for the diagnosis of tuberculous meningitis.¹¹ Assuming the sensitivity of Xpert was 60%,¹⁸ using a significance level of 5% and 80% power, we calculated that 49 patients with tuberculous meningitis were required in each of the testing groups to be able to detect a 25% difference in sensitivity. To provide robust specificity estimates, CSF from at least 100 patients with non-tuberculous meningitis central nervous system infections was also tested.

Univariable and multivariable logistic regression analyses were done to identify factors associated with microbiological confirmation (ie, positive smear, Xpert, Xpert Ultra, or MGIT) of tuberculous meningitis. The following variables were tested: age, sex, duration of illness, Glasgow Coma Score, Medical Research Council tuberculous meningitis grade, CSF–blood glucose ratio, CSF lactate, CSF protein, CSF lymphocyte percentage, and CSF volume.

Statistical analysis was done using the programming language R (version 3.5.1).

Role of the funding source

The funders had no role in study design, data collection, data analysis, data interpretation, writing of the report, or the decision to submit the paper for publication. The corresponding author had full access to all of the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Oct 16, 2017, and Feb 10, 2019, 205 participants were consecutively enrolled into the study and randomly assigned to Xpert Ultra (n=103) or Xpert (n=102; figure 1). Of the 204 participants who obtained a final diagnosis, as per the uniform case definition for tuberculous meningitis,⁸ 82 (40%) were diagnosed with definite, six (3%) with probable, 20 (10%) with possible, and 96 (47%) with not tuberculous meningitis (figure 1; table 1). Baseline variables, including age and sex, in the Xpert Ultra and Xpert groups seemed well matched (table 1). Median CSF volumes used for mycobacterial testing were similar in both groups (table 1). Median time to MGIT positivity was 15 days (IQR 10–18) in the

	Xpert Ultra (n=103)	Xpert (n=102)
Age (years)	42 (31–57)	44 (33–58)
Sex		
Female	41 (40%)	35 (34%)
Male	62 (60%)	67 (66%)
Final diagnosis		
Definite tuberculous meningitis	44 (43%)	38/101 (38%)
Probable tuberculous meningitis	1 (1%)	5/101 (5%)
Possible tuberculous meningitis	10 (10%)	10/101 (10%)
Not tuberculous meningitis	48 (47%)	48/101 (48%)
Medical Research Council tuberculous meningitis grade*		
1	19/55 (35%)	13/53 (25%)
2	22/55 (40%)	25/53 (47%)
3	14/55 (25%)	15/53 (28%)
HIV status		
Positive	17 (17%)	14/99 (14%)
Negative	48 (47%)	48/99 (48%)
Unknown	38 (37%)	37/99 (37%)
CSF characteristics*		
CSF white cell count (per µL)	310 (172–597); n=55	334 (120–484); n=53
CSF lymphocytes	76% (38–88); n=55	74% (41–86); n=52
CSF protein (g/L)	1.87 (1.12–2.79); n=55	1.96 (1.31–2.92); n=53
CSF blood glucose ratio	0.35 (0.22–0.46); n=55	0.35 (0.25–0.43); n=53
CSF volume for mycobacterial tests (mL)	5.8 (5.0–6.0); n=100	5.5 (5.0–6.0); n=101
Time to CSF MGIT positivity (days)†	15 (10–18); n=23	18 (13–20); n=22

Data are n (%), n/N (%), or median (IQR); number assessed is listed with the median when not assessed in all patients. Xpert Ultra=Xpert MTB/RIF Ultra. Xpert=Xpert MTB/RIF. CSF=cerebrospinal fluid. MGIT=mycobacteria growth indicator tube. *Medical Research Council grade and CSF characteristics shown only for 108 patients with definite, probable, or possible tuberculous meningitis, with the exception of CSF volume for mycobacterial tests. †Data shown for all 45 CSF samples for which MGIT was positive.

Table 1: Baseline characteristics

Xpert Ultra group and 18 days (13–20) in the Xpert group. Disease severity, HIV status, and CSF parameters also appeared well matched between the two groups (table 1).

The diagnostic sensitivities of Xpert Ultra and Xpert against the reference standard of definite, probable, and possible tuberculous meningitis were 47.2% (95% CI 34.4–60.3) for Xpert Ultra and 39.6% (27.6–53.1) for Xpert ($p=0.56$; table 2). Specificities of Xpert Ultra and Xpert were both 100.0% (table 2). Against a mycobacterial culture reference standard, sensitivities were 90.9% (95% CI 72.2–97.5; 20 of 22) for Xpert Ultra and 81.8% (61.6–92.7; 18 of 22) for Xpert and specificities were

	Xpert Ultra	Xpert	Ziehl-Neelsen smear	MGIT culture
Reference standard: definite, probable, and possible tuberculous meningitis				
Positive tests	25/53*	21/53	77/108	45/94
Sensitivity	47.2% (34.4–60.3)	39.6% (27.6–53.1)	71.3% (62.5–79.0)	47.9% (38.0–57.9)
Specificity	100.0% (92.0–100.0)	100.0% (92.6–100.0)	100.0% (96.1–100.0)	100.0% (95.6–100.0)
PPV	100.0% (86.7–100.0)	100.0% (84.5–100.0)	100.0% (95.2–100.0)	100.0% (92.1–100.0)
NPV	61.1% (49.6–71.5)	60.0% (49.0–70.0)	72.2% (67.2–82.1)	63.2% (54.7–70.9)
Reference standard: definite and probable tuberculous meningitis				
Positive tests	25/43	21/43	77/88	45/75
Sensitivity	58.1% (43.3–71.6)	48.8% (34.6–63.2)	87.5% (79.0–92.9)	60.0% (48.7–70.3)
Specificity	100.0% (93.4–100.0)	100.0% (93.8–100.0)	100.0% (96.8–100.0)	100.0% (96.4–100.0)
PPV	100.0% (86.7–100.0)	100.0% (84.5–100.0)	100.0% (95.2–100.0)	100.0% (92.1–100.0)
NPV	75.0% (63.9–83.6)	72.5% (61.9–81.1)	91.3% (85.0–95.1)	77.4% (69.6–83.7)
Reference standard: definite tuberculous meningitis				
Positive tests	25/42	21/38	77/82	45/73
Sensitivity	59.5% (44.5–73.0)	55.3% (39.7–69.9)	93.9% (86.5–97.4)	61.6% (50.2–72.0)
Specificity	100.0% (93.5–100.0)	100.0% (94.3–100.0)	100.0% (96.3–100.0)	100.0% (96.5–100.0)
PPV	100.0% (86.7–100.0)	100.0% (84.5–100.0)	100.0% (95.2–100.0)	100.0% (92.1–100.0)
NPV	76.4% (65.4–84.7)	78.8% (68.6–86.3)	95.3% (89.4–98.0)	79.0% (71.3–85.0)

Data in parentheses are 95% CIs. Xpert Ultra=Xpert MTB/RIF Ultra. Xpert=Xpert MTB/RIF. MGIT=mycobacteria growth indicator tube. PPV=positive predictive value. NPV=negative predictive value. *Of 55 cases of definite, probable, or possible tuberculous meningitis tested by Xpert Ultra, two with definite tuberculosis returned an error result. Therefore, only 53 cases are included in the sensitivity calculation. Of the 205 participants enrolled in the study, Ziehl-Neelsen smear and MGIT were done in 204 (>99%) and 187 (91%) cases, respectively.

Table 2: Diagnostic performance of Xpert Ultra and Xpert against clinical reference standard

93.9% (85.4–97.6; 62 of 66) for Xpert Ultra and 96.9% (89.5–99.2; 63 of 65) for Xpert. The sensitivities of Xpert Ultra and Xpert were similar against reference standards of definite and probable tuberculous meningitis (Xpert Ultra vs Xpert $p=0.52$) and definite tuberculous meningitis ($p=0.87$). Sensitivities were 59.5% (44.5–73.0; 25 of 42) for Xpert Ultra and 55.3% (39.7–69.9; 21 of 38) for Xpert against the reference standard of definite tuberculous meningitis ($p=0.87$; table 2). Neither Xpert Ultra nor Xpert was as sensitive as Ziehl-Neelsen smear against any reference standard (table 2). Ziehl-Neelsen smear was significantly more sensitive than both Xpert Ultra and Xpert (data not shown), as shown in previous papers.^{1,18}

When considering the distribution and overlap of positive CSF by Xpert Ultra, Xpert, Ziehl-Neelsen smear, and MGIT, all positive Xpert Ultra or Xpert cases were also positive by Ziehl-Neelsen smear, MGIT, or both (figure 2). There were six error results with Xpert Ultra and none with Xpert. Eight MGIT samples showed contaminated growth. Tested CSF volumes did not vary substantially (median 5.8 mL, IQR 5.0–6.0, in all patients combined; table 1), but CSF volume did not appear to influence the likelihood of a positive Xpert Ultra or Xpert test (appendix p 1). Univariable and multivariable analysis of factors predicting microbiological confirmation of tuberculous meningitis are shown in the appendix (p 2). Female sex was associated with a reduced likelihood of microbiological confirmation of tuberculous meningitis in both univariable and multivariable analyses,

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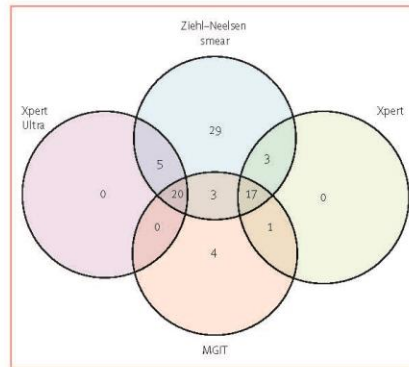


Figure 2: Positive mycobacterial tests in individuals with at least one confirmatory test for tuberculous meningitis. 168 positive mycobacterial tests (25 positive by Xpert Ultra, 21 by Xpert, 77 by Ziehl-Neelsen smear, and 45 by MGIT) from 82 patients with a diagnosis of definite tuberculous meningitis. MGIT=mycobacteria growth indicator tube. Xpert Ultra=Xpert MTB/RIF Ultra. Xpert=Xpert MTB/RIF.

	Xpert Ultra		Xpert	
	HIV negative	HIV positive	HIV negative	HIV positive
Reference standard: definite, probable, and possible tuberculous meningitis				
Positive tests	14/36	9/14	8/35	10/13
Sensitivity	38.9% (24.8–55.1)	64.3% (38.8–83.7)	22.9% (12.1–39.0)	76.9% (49.7–91.8)
Specificity	100.0% (70.1–100.0)	100.0% (43.9–100.0)	100.0% (77.2–100.0)	100.0% (20.7–100.0)
PPV	100.0% (78.5–100.0)	100.0% (70.1–100.0)	100.0% (67.6–100.0)	100.0% (72.2–100.0)
NPV	29.0% (16.1–46.6)	37.5% (13.7–69.4)	32.5% (20.1–48.0)	25.0% (4.6–69.9)
Reference standard: definite and probable tuberculous meningitis				
Positive tests	14/29	9/11	8/27	10/12
Sensitivity	48.3% (31.4–65.6)	81.8% (52.3–94.9)	29.6% (15.9–48.5)	83.3% (55.2–95.3)
Specificity	100.0% (80.6–100.0)	100.0% (61.0–100.0)	100.0% (84.5–100.0)	100.0% (34.2–100.0)
PPV	100.0% (78.5–100.0)	100.0% (70.1–100.0)	100.0% (67.6–100.0)	100.0% (72.2–100.0)
NPV	51.6% (24.8–68.0)	75.0% (40.9–92.9)	52.5% (37.5–67.1)	50.0% (15.0–85.0)
Reference standard: definite tuberculous meningitis				
Positive tests	14/28	9/11	8/23	10/11
Sensitivity	50.0% (32.6–67.4)	81.8% (52.3–92.9)	34.8% (18.8–55.1)	90.9% (62.3–98.4)
Specificity	100.0% (80.6–100.0)	100.0% (61.0–100.0)	100.0% (84.5–100.0)	100.0% (43.9–100.0)
PPV	100.0% (78.5–100.0)	100.0% (70.1–100.0)	100.0% (67.6–100.0)	100.0% (72.2–100.0)
NPV	53.3% (36.1–69.8)	75.0% (40.9–92.9)	58.3% (42.2–72.9)	75.0% (30.1–95.4)

Xpert Ultra=Xpert MTB/RIF Ultra. Xpert=Xpert MTB/RIF. PPV=positive predictive value. NPV=negative predictive value.

Table 3: Diagnostic performance of Xpert and Xpert Ultra against clinical reference standard, by HIV status

with all other factors non-predictive in the multivariable analysis.

HIV testing was done in 127 (63%) of 202 participants and 100 (93%) of 108 participants with at least possible tuberculous meningitis. 31 patients were HIV co-infected (17 [26%] of 65 cases in the Xpert Ultra group and 14 [23%] of 62 cases in the Xpert group; table 1). In HIV-uninfected participants, Xpert Ultra was not more sensitive than

Xpert against the reference standard of definite, probable, and possible tuberculous meningitis ($p=0.23$), nor against the reference standard of definite and probable tuberculous meningitis ($p=0.25$) or definite tuberculous meningitis ($p=0.42$; table 3). Both tests were 100% specific in this patient group (table 3).

In HIV co-infected participants, the two tests performed similarly against a reference standard of definite and probable tuberculous meningitis (table 3). Against definite, probable, and possible tuberculous meningitis, the sensitivities were 64.3% (95% CI 38.8–83.7) for Xpert Ultra and 76.9% (49.7–91.8) for Xpert ($p=0.77$; table 3). Against definite and probable tuberculous meningitis, the sensitivities were 81.8% (95% CI 52.3–94.9; nine of 11) for Xpert Ultra and 83.3% (55.2–95.3; ten of 12) for Xpert ($p=1.0$). Specificities of Xpert Ultra and Xpert were both 100% (table 3). Against a mycobacterial culture reference standard, sensitivities of Xpert Ultra and Xpert in HIV-uninfected participants were 83.3% (55.2–95.3; ten of 12) for Xpert Ultra and 60.0% (31.3–83.2, six of ten) for Xpert ($p=0.55$), and in HIV co-infected participants sensitivities were 100.0% (95% CI 70.1–100.0; nine of nine) for Xpert Ultra and 100.0% (70.1–100.0; nine of nine) for Xpert ($p=1.0$).

Xpert can categorise specimen bacterial numbers into high, medium, low, or very low. Xpert Ultra has an additional trace category. The categories obtained from the CSF are shown in the appendix (p 5). The number of samples with medium or low numbers of bacteria were similar between the two groups (ten for Xpert Ultra and 13 for Xpert), suggesting similar baseline bacterial concentrations in the two patient groups. 15 (60%) of 25 CSF samples positive by Xpert Ultra were categorised as containing very low or trace numbers of bacteria compared with eight (38%) of 21 samples with very low bacterial numbers detected by Xpert.

Rifampicin resistance was detected in eight (17%) of 46 positive tests: five (20%) of 25 positive tests by Xpert Ultra and three (14%) of 21 positive tests by Xpert. All five cases categorised as trace positive by Xpert Ultra returned a result of indeterminate resistance. Rifampicin resistance testing was negative in 22 (72%) cases where either Xpert Ultra or Xpert were positive. Of 45 patients with positive CSF MGIT cultures, eight showed rifampicin resistance by phenotypic drug susceptibility testing, all of which were detected by Xpert Ultra ($n=5$) or Xpert ($n=3$).

Routine follow-up CSF was sampled and tested in 49 patients treated for tuberculous meningitis (27 by Xpert Ultra and 22 by Xpert). A median of 5.5 mL (IQR 5.0–6.0) CSF was tested in each of the groups. 13 (48%) patients in the Xpert Ultra group and eight (36%) in the Xpert group had a positive test at baseline. After a mean of 27 days (SD 5.9) of antituberculosis treatment in the Xpert Ultra group and 28 days (SD 5.4) in the Xpert group, six (22%) participants in the Xpert Ultra group had a positive test versus two (9%) in the Xpert group (figure 3). Restricting the analysis to those positive

by Xpert Ultra or Xpert at baseline, five (38%) of 13 patients in the Xpert Ultra group were still positive after 3–4 weeks' treatment, compared with two (25%) of eight patients in the Xpert group. The influence of drug resistance on a positive test by Xpert Ultra or Xpert at 3–4 weeks after treatment initiation is shown in the appendix (p 3). Median CSF parameters at repeat diagnostic testing in individuals with positive and negative NAATs at repeat testing are also shown in the appendix (p 4), compared with baseline CSF parameters.

Discussion

In our study, Xpert Ultra was not superior to Xpert for the detection of *M tuberculosis* in CSF of individuals with tuberculous meningitis, using either clinical or culture reference standards. Moreover, Xpert Ultra was not more sensitive than Xpert in HIV-uninfected individuals when compared against all variations of the clinical tuberculous meningitis reference standard or against mycobacterial culture. The sensitivity of both assays was higher in HIV-infected than in HIV-uninfected individuals, probably reflecting the larger numbers of bacteria in CSF samples from these patients.¹⁹ Additionally, Xpert Ultra appeared to be able to detect more patients with tuberculous meningitis with very low or trace levels of bacteria in their CSF and to be more sensitive than Xpert once antituberculosis treatment had been started.

Previous studies have suggested that Xpert Ultra is significantly more sensitive than Xpert for the diagnosis of tuberculous meningitis.^{12,13} In their cohort of 23 HIV co-infected patients with definite or probable tuberculous meningitis, Bahr and colleagues reported sensitivities of 69.6% for Xpert Ultra and 43.5% for Xpert, compared with 81.8% and 83.3%, respectively, in the HIV co-infected patients included in our study. What might be the explanation for these different results? First, we tested fresh as opposed to stored, frozen CSF samples, which could impair or alter the performance of the assays. Second, we tested the maximum volume of CSF available with one assay rather than dividing the sample in two for simultaneous testing with both assays, which would reduce the number of bacteria available for detection and could reduce the sensitivity of both assays. It is plausible the effect of CSF volume on performance is greater for Xpert than for Xpert Ultra, which can detect trace numbers of bacteria. This possible threshold effect might explain why Xpert Ultra had a higher sensitivity than Xpert for *M tuberculosis* detection in the CSF of HIV-uninfected individuals in our study, albeit without reaching significance, but with no apparent difference between Xpert and Xpert Ultra performance in HIV co-infected tuberculous meningitis. The number of CSF bacteria in HIV-uninfected individuals with tuberculous meningitis could be very close to or below the detection threshold of Xpert but above that of Xpert Ultra, which might drive the difference in performance. HIV co-infected patients have greater numbers of CSF

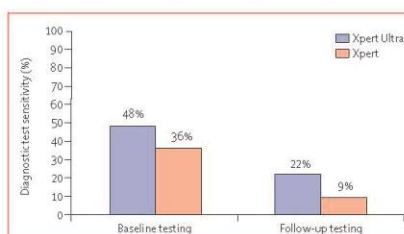


Figure 3: Comparison of the diagnostic sensitivities of Xpert Ultra and Xpert between baseline and testing after 4 weeks of antituberculosis treatment. Data shown for 49 patients (27 Xpert Ultra, 22 Xpert) undergoing both baseline and follow-up CSF sampling. All cases with a positive nucleic acid amplification test at follow-up testing ($n=8$) were positive at baseline testing. Xpert Ultra–Xpert MTB/RIF Ultra, Xpert–Xpert MTB/RIF.

bacteria than do HIV-uninfected patients and, therefore, samples from these patients might exceed the detection thresholds of both assays when large volumes are tested. This CSF bacterial load could account for why test performances in HIV infection appeared more closely aligned than in HIV-uninfected patients. These hypotheses would be supported by showing a correlation between the performance of each assay and CSF volume tested. However, in our study, there was too little variation in the volumes tested to be able to define a correlation with performance of either assay. Therefore, these explanations remain speculative. Finally, the mean copy number of the multicopy amplification target IS6110 varies between *M tuberculosis* of different lineages,²⁰ which might affect Xpert Ultra detection of *M tuberculosis* in different countries. However, isolates belonging to the L2 lineage (the predominant lineage found in Vietnam²¹) have the highest mean copy number of IS6110,²⁰ which in theory would improve the diagnostic sensitivity of Xpert Ultra at our site, whereas the L4 lineage (predominant in Africa) shows large variation in IS6110 mean copy number.²²

The specificity of Xpert Ultra and Xpert in our study was 100% when clinical reference standards were used. The Xpert Ultra specificity decreased slightly only when MGIT culture was used as a reference standard. In a previous study wherein Xpert Ultra was used to test sputum from patients with suspected pulmonary tuberculosis, Xpert Ultra showed reduced specificity compared with Xpert; however, mycobacterial culture was the reference standard used in that study.¹¹ A decrease in NAAT specificity is expected when a reference standard of mycobacterial culture is used; culture will only detect viable bacteria, whereas NAAT might detect DNA of dead bacteria that cannot be cultured, leading to apparent false-positive NAAT results against a mycobacterial culture reference standard.²³

In our study, there were 25 positive Xpert Ultra tests from 55 tested patients with definite, probable, or

possible tuberculous meningitis. All 25 had a positive Ziehl–Neelsen smear and 20 (80%) had a positive MGIT. No positive Xpert Ultra results were recorded in patients with a probable or possible diagnosis of tuberculous meningitis, nor in any patient in whom a non-tuberculous meningitis diagnosis was confirmed. In our setting, Xpert Ultra did not diagnose additional cases of tuberculous meningitis missed by other confirmatory mycobacterial testing methods, probably due to the high sensitivity of Ziehl–Neelsen smear microscopy at our site, which has been consistently high over many years.^{18,24} The sensitivity of Ziehl–Neelsen smear at our site repeatedly exceeds that of Xpert and MGIT.¹⁸ At sites where CSF smear and mycobacterial culture have lower sensitivity, Xpert Ultra could provide more value. At our site, Ziehl–Neelsen smear slides are meticulously examined for 30 min by skilled technicians experienced at identifying acid-fast bacteria in CSF. Centrifugation of CSF at 3000 g for 15 min, resuspension of CSF pellet by vortexing with the sample reagent, and use of 100 µl (20%) of this resuspended CSF pellet for Ziehl–Neelsen smear also improve the diagnostic performance of this test.²⁵

The strengths of this study are that it is large, prospective, and randomised, and includes data on the performance of both tests after the start of antituberculosis treatment. The use of randomisation to a single test (ie, with each CSF sample tested by Xpert Ultra or Xpert, not both) is a strength as division of the CSF pellet to allow both Xpert Ultra and Xpert testing to be done on a single CSF sample does not reflect normal clinical practice and could reduce the sample bacillary load and diagnostic sensitivity of both tests. Additionally, laboratory technicians were masked to the patients' clinical characteristics on baseline testing, and testing was done immediately after randomisation. CSF was sampled, processed, and tested in the same way for both Xpert Ultra and Xpert. With the high sensitivity of smear at our site, we were able to microbiologically confirm a high number of tuberculous meningitis cases and, together with MGIT, show that all positive Xpert Ultra tests were true positives.

A limitation of our study is that specificity estimates are mostly from HIV-uninfected individuals; additional data are required to confirm a high specificity of Xpert Ultra in HIV co-infection. Additionally, although individuals with tuberculous meningitis underwent routine HIV testing in our study, HIV testing was not mandatory for all study patients. Another limitation of our study is that, although our study was powered to detect a 25% improvement in diagnostic sensitivity with Xpert Ultra compared with Xpert, we cannot conclude whether Xpert Ultra is superior to Xpert at a lower margin of superiority given our study was not powered to detect smaller differences. Likewise, our study was not powered to detect performance differences in subgroups defined by HIV. Although our randomised study design has strengths, as described above, a limitation with this design is that there is a greater

possibility of imbalance between groups than with a design where both diagnostic tests are done on a single CSF sample. There was, however, no evidence of imbalance in any important parameters between the randomisation groups. Additionally, the diagnostic performance of Xpert Ultra compared with Xpert might have been higher than measured in our study because of a higher proportion of individuals randomly assigned to Xpert Ultra than to Xpert having low or trace results.

In summary, the performances of Xpert Ultra and Xpert for the diagnosis of tuberculous meningitis were similar. Although the sensitivity of Xpert Ultra was higher than that of Xpert in patients without HIV infection, this difference was not significant. Our results suggest that Xpert Ultra might perform better than Xpert in patients without HIV infection and remain positive for longer after the start of antituberculosis treatment. Xpert Ultra testing could be preferable when CSF bacillary load is low. However, neither Xpert Ultra nor Xpert have a sufficiently high negative predictive value to rule out tuberculous meningitis. Therefore, the search must continue for a better diagnostic test for tuberculous meningitis.

Contributors

JD contributed to study design, data analysis, and manuscript drafting, editing, and writing. DDAI, VTMD, and TPQ contributed to laboratory testing and collection of laboratory data. NHP, HDTN, PKNO, and TBN contributed to collection of clinical data. NVVC contributed to study concept and manuscript editing. VTNH contributed to laboratory quality control and laboratory testing. VTTH contributed to data collection and analysis. DHKT contributed to statistical analysis. RBG contributed to statistical analysis and manuscript editing. LVT contributed to study design. NITT contributed to study design, laboratory supervision, and manuscript editing. GET contributed to study concept and design and manuscript editing and writing.

Declaration of interests

We declare no competing interests.

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